PB83-217802

Use of Microcosms for Evaluation of Interactions between Pollutants and Microorganisms

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

Jun 83

U.S. Department of Commerce
National Technical Information Service
NTIS

PB83-217802

EPA-600/D-83-050
June 1983

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/D-83-050

2.

3. RECIPIENT'S ACCESSION NO.
PB83 217802

4. TITLE AND SUBTITLE
THE USE OF MICROCOSMS FOR EVALUATION OF INTERACTIONS BETWEEN POLLUTANTS AND MICROORGANISMS

5. REPORT DATE
June 1983

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
P.H. Pritchard and A.W. Bourquin

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Office of Research and Development
Gulf Breeze, FL 32561

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Microcosms or modern ecosystem studies are described and evaluated as tools to assess the response of a microbial community to a pollutant released in the environment. Precautions and potentials are discussed regarding the potential role of microcosms in risk assessment of environmental hazards, and their capability to predict the fate and effects of organic compounds in aquatic environments.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
Release to public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
137

22. PRICE

NOTICE

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1. Introduction

Experimental studies of the interactions within microbial communities has been a standard banner of microbial ecologists for many years. Their emphasis has been to bridge the gap between pure culture studies in the laboratory and field observations in natural ecosystems. Concern over the long-term effects of pollution on ecosystem processes has continuously challenged existing knowledge about the types, rates, and extents of these interactions, including their resistance and resilience to a large array of man-made perturbations.

The resulting interplay between research in microbial ecology and the efforts to understand the fate and effects of xenobiotics in natural systems has progressed from experimental studies with pure cultures to more complex studies using mixed cultures and communities of microorganisms (Bull, 1980; Bourquin and Pritchard, 1980). Laboratory studies with communities of microorganisms have not been particularly dificult experimentally; a large variety (but not necessarily a large number) of such studies has been generated (e.g., see Bourquin and Pritchard, 1980). This progression has been accompanied by increased concern about the "naturalness" of microbial communities studied in the laboratory. Specifically, the concern centers around the environmental significance of information derived from mixed culture or multispecies studies; i. e., are the culture conditions in standard shake flasks or in completely mixed reactors containing an inoculum of some environmental sample reflective of conditions in nature, or can laboratory systems maintain the physical integrity of natural systems and model natural interactions of microbial communities with higher trophic levels? In response, microbial ecologists, among other ecologists and toxicologists, have developed a variety of unique and practical experimental methods that address the question of how laboratory-derived data can be used in predicting potential deleterious impacts of general and specific pollution events to natural environments. An example of such a development has been the appearance of "microcosms" or "model ecosystems" studies in the areas of microbiology, ecology, and toxicology. The term "microcosm" is undoubtedly the result of a whimsical indulgence in semantics because its literal application to biology borders on absurdity. But it is a term that is used in the scientific literature with increasing frequency, and has been considered a valuable descriptor of certain laboratory studies (e.g., see Giesy, 1980). Like many words of similar, nonspecific origin, however, it has a "pseudocognate" meaning; that is, each user feels that his audience share his own intuitive definition (Salt, 1979). This situation, if not corrected by a formal definition of the word, will lead to cynicism, ambiguity, and eventually the loss of a word that could stimulate new and valuable experimental concepts.

For the purposes of this paper, a microcosm study is defined as an attempt to bring an intact, minimally disturbed piece of an ecosystem into the laboratory for study in its natural state. It is the establishment of a physical model or simulation of part of the ecosystem in the laboratory within definable physical and chemical boundries under a controlled set of experimental conditions (i. e., lighting, humidity, aeration, mixing, temperature). The design of the microcosm is a function of the question under study; the boundry conditions of a microcosm can be manipulated for the purposes of testing hypotheses and thereby draw inferences on causality and mechanisms of interaction.

A microcosm is theoretically representative of the level of complexity found in the portion of the ecosystem from which it was sampled. Microcosms should, therefore, be self-sustaining to a large extent and should demonstrate the same homeostasis as natural ecosystems. Since this level of complexity goes beyond simple composites or mixed cultures of microorganisms (most mixed culture studies are not necessarily microcosm studies), the principle emphasis for the microbial ecologist is the term "simulate" or "intact," which distinguish a microcosm study from common mixed culture or bacterial community studies. The attempt in a microcosm study is to avoid the arbitrary establishment of an isolated bacterial community or a mixed culture interaction in the laboratory. Instead, an intact portion of the environment is transported to the laboratory and maintained in conditions as close to real-life as possible, preserving both the physical integrity of the environmental sample and the interactions of many communities at several levels of trophic organization. Relationships between the activities of microbial communities and bioturbation by invertebrate meiofauna in sediment, grazing by organisms that depend on bacteria as food sources, the exudates of plant surfaces and root systems, or phytoplankton/zooplankton community dynamics, would appropriately be studied in microcosms. The complexity of such studies is enormous, particularly when one considers the limited experimental knowledge of the environmental factors which control the activities of isolated microbial communities. In the long term, however, a more productive approach to the study of (microbial) ecology may result (Hill and Weigert, 1980).

Microcosms may be considered as analogs to the field; thus, studies with a microcosm are surrogates to actual field studies. As such, microcosms can be dosed with a pollutant in lieu of dosing the field, this potentially circumventing the logistical, financial, and administrative problems associated with field exposures. This dosing capability is the most important attribute of and justification for research with microcosms. They should be consequently very useful in aiding and supplementing decisions about real and potential impacts of toxic organic materials in nature.

A relatively large amount of microcosm literature (some microbiological) has recently been summarized (Witt and Gillette, 1977, Giesy, 1980; Pritchard, 1981, Levendowsky, 1977, draggon, 1979.)

A review of microcosm studies is basically a methodological consideration. Microcosms do not represent a discipline into themselves but instead can be applied in different ways to a wide variety of microbiological studies. Like other research methods, microcosms have certain limits and criteria for their application and for the interpretation of the results derived in their use. Much of the foundation for these applications and conceptualizations has come from microcosm studies in which bacterial processes are considered either indirectly or not at all. However, the experience gained in using the microcosm method in a variety of ecological contexts is quite revelant to microbial ecology investigations. A number of microbiological undertakings, of course, have used microcosm-like methods and concepts without formal mention of the term.

The following aspects of microcosm-related research are considered:

a. The microcosm approach - how microcosms offer specific advantages over field studies.

b. Their role in risk assessment - microcosms can represent an important part of the evaluation of environmental hazard by certain pollutants if used in conjunction with mixed culture (multispecies) and pure culture (single species) studies.

c. Qualitative predictors of toxic chemical fate and effects - microcosms can contribute to screening programs for pollutant fate and effects by providing a routine test system with ecosystem complexity.

d. Quantitative predictors of microbial processes - microcosm studies help elucidate the mechanisms, kinetics, and controlling variables of microbially mediated process in a variety of complex ecosystem compartments and they can help determine the environmental relevancy of data produced from simpler laboratory test methods. Specific treatments of a microcosm, judicious intergration with mixed culture studies, and interpretation of results through the use of mathematical models must be undertaken.

e. Physical integrity of natural ecosystems - It is argued that a proper assessment of the interactions between pollutants and microorganisms in aquatic environments must take into account the natural physical integrity of ecosystem components, e.g., sediment/water interfaces, redox gradients. A microcosm is the principal method for maintaining this integrity in the laboratory.

f. Interaction with higher trophic levels - It is argued that microbial responses to pollutants are tightly coupled to the interplay between microbial communities and higher organisms (grazers, phytoplankton, fungi, etc.). Microcosms provide the best laboratory means of studying these responses.

g. Field calibration - The successful use of a microcosm requires that it simulates a natural field situation. Various ecosystems functions and structures can be used in this calibration exercise.

h. Precautions and potentials - Microcosms have certain significant limitations but their potential as a productive and informative laboratory method is considered to be excellent.

Our discussion and citations carry a certain bias in that they reflect the authors' interests in biodegradation and aquatic ecosystems (particularly estuarine).

2. History of the Microcosm Research Approach: Environmental Risk Assessment.

The need for microcosm studies originated from two important considerations: the fear of simplicity and the problems associated with using pollutants and xenobiotics in field studies. The fear of oversimplification has been a common problem in the approach to standardized tests for assessing the potential impacts of a toxicant to an ecosystem or to a microbial community. Often, this approach has involved single species or pure cultures whose isolation from the complexities of nature has cast considerable doubt on environmental significance of the test results. As microbiologists have recognized that the toxicity of a chemical to a pseudomonad or the degradation of a chemical by a pure culture is of little quantitative value in assessing impacts in the field, toxicologists have also recognized the need to complement single-species bioassay tests with toxicological studies on communities of organisms. The justification has been that the interactions among populations may be more sensitive to toxicants and that community studies may ultimately lead to a more quantitative estimation of ecosystem effects. The microcosm is preceived as a potential laboratory tool which could help reduce this "fear of simplicity" by permitting fate and effects studies in much more ecologically complex laboratory systems. Whether this approach will, in fact, relieve this fear depends on how microcosm results are interpreted.

Problems encountered in carrying out field studies with toxic or persistent pollutants were the second major factor behind the development of microcosms. Although scientists would like to ultimately measure the fate and effects of a pollutant by dosing a natural system (a field site), the opportunities for such experiments are extremely limited. The risk of environmental damage is a social and regulatory problem that is difficult to reconcile. In addition, the enormous problems of setting up the enclosure, mixing the chemical after dosing, and adequately sampling for fate and effects information are well recognized (Steel and Menzel, 1978; Case, 1978).

Several attempts have been undertaken, for example, to enclose large portions of various aquatic ecosystems to study the fate and effects of such compounds as polycyclic aromatic hydrocarbons (Lee *et al.*, 1978) and heavy metals (Beers *et al.*, 1977; Davis and Gamble, 1979). These studies have used very large polyethylene bags (60,000- to 170,000-liter capacity) to trap parts of an ecosystem in the field. The MERL tank at the University of Rhode Island (Pilson *et al.*, 1980; Pilson *et al.*, 1979) is a similar type of enclosure system except the tanks are land-based, and seawater and sediment must be added to the tanks. Ecosystem parameters measured in enclosure experiments generally show good correlation with and similarity to actual field measurements. However, their cost and labor intensity restrict their application to a variety of pollutants. It is also not clear how much of the information gained from enclosure experiments might have also been obtained in smaller and more convenient systems in the laboratory.

If a section of the environment could be brought into the laboratory (as a microcosm), and shown to behave as in the field, then it would offer a more practical system to dose with a toxicant. It would provide a number of significant advantages over a field study. First, the recognition of a particular toxic effect or fate phenomena could be greatly enhanced since natural variations in functional and structural parameters caused by seasonal and climatic events could be minimized. This assumes that a microcosm operated under a standard set of laboratory conditions will still serve as a reasonable analog or surrogate to the field. It also assumes that results obtained from the microcosm could be extrapolated to the field over large spans of space and time. Various studies indicate that these assumptions are correct (Harte _et al_., 1980; Hill and Weigert, 1980; Schindler, et al., 1980).

Second, their small size permits replication, simplified dosing mechanics, control over imports and exports from the system, and adequate mixing, all factors which are difficult to control in the field. The microcosm gives the researcher the opportunity to vary environmental conditions to simulate a large array of possible perturbations to the system. These options are not available in field studies. If the proper concepts and logic can be developed to extrapolate microcosm results to the field, then the methodology has great promise for understanding the interactions of natural communities, both at the microbial level and at higher trophic levels.

3. Role of Microcosms in Environmental Risk Assessment

Information from laboratory tests can be used to evaluate the behavior of a chemical in the environment in a qualitative or screening type assessment. Toxicity testing, for example involves a determination of the concentration at each test chemical will elicit a specific effect, such as mortality, decreased growth rate, decreased metabolic activity, using the same highly standaradized bioassay test. Biodegradability testing involves the measurement of the extent or rate in which a chemical is degraded, using standardized measures of biological oxygen demand, mineralization, or loss of parent compound, and a standard inoculum such as sewage sludge. In both cases, risk assessment is based on a relative comparison of test results in which the test information from one chemical is ranked within a data base for a large array or previously tested chemicals, by a standardized method. The test methods do not necessarily produce environmentally realistic data but rather, "worst case" situations. The emphasis is on consistency of results and method of standardization. Decisions on which chemicals are considered a potential hazard to the environment are based on scientific prudence, common sense, and consensus judgments.

The second use of laboratory tests in evaluating the behavior of a chemical in the environment is quantitative assessment, or a determination of waste assimilatory capacity [the potential of any environment to accept pollution without adverse effects (Cairns, 1981)]. An assessment of this type, of course, requires accurate measures of the rates and dynamics of both fate and effects processes, including their interactions, and a determination of how toxic chemicals will quantitatively alter these processes. It requires answers to questions such as: 1) how much of a reduction in a microbial population density, activity, or diversity can be tolerated before permanent damage (based on some carefully qualified time scales) occurs; 2) how much of a response is required before ecosystem balance is upset; or 3) how much of a difference in biodegradation rates of a chemical is measured before the chemical is considered persistent in one environmental area and not in another?

Microcosms can be potentially used in both screening programs and waste assimilatory capacity determinations. However, Figure 1 is a diagrammatic representation of how information from laboratory fate and effects tests (including microcosms) is currently applied to relative (screening) assessments and to waste assimilatory capacity determinations. Fate and effects testing is divided into three levels of organization: 1) single species tests which use pure cultures of bacteria and other organisms, 2) multispecies or mixed culture tests which use inocula from the field in an experimentally convenient manner, and 3) microcosm tests which use intact field samples from the environment.

Mixed culture tests ignore any controlling factors which might result from taking intact field samples and maintaining them in the laboratory under natural field conditions or which might result from isolating a bacterial community from interactions with other communities (plant, animal, and bacterial) or trophic structures. These tests generally have a minimal number of physical components. The emphasis in complexity is confined to mixtures of species or isolated communities and does not usually include relationships with other components in the ecosystem.

Microcosm tests, on the other hand, are basically more rigorously mixed culture tests, involving a number of physical components and involving studies of environmental samples in the laboratory where the natural physical integrity of the field sample is maximized and isolation from other ecosystem components is minimized.

3.1) Single-species Tests

Single-species tests primarily support relative assessments or screening programs. This is particularly true of the single-species bioassays used in many areas of toxicology. Little of this information can be applied to quantitative assessments. In many cases, however, the response of a single species to a particular chemical may provide invaluable mechanistic information which can be applied to the interpretation of a more complex experimental system. Pure cultures of bacteria can of course be used to examine in detail the toxicity of certain chemicals; for example, the effects of chlordane on *Bacillus*, *Streptoccus*, and *Mycobacterium* (Widdus *et al*., 1971). They can be used to examine the mechanisms by which bacteria degrade specific chemicals; for example, mechanisms of cometabolism and biodegradation of DDT (Pfaender and Alexander, 1972). Although quantitative information on fate and effects processes (such as rates of biodegradation or extents of metabolic inhibition) can be obtained from single species tests, it has been pointed out (Bull, 1980; Tempest, 1970; Levin, 1982) that the measurements cannot easily be applied to natural field situations because the test organisms are extensively laboratory-acclimated and experimental conditions provide for optimized growth and survival, a condition unlikely to be found in the field. This is why, in many respects, "worst-case" interpretations have been invoked.

3.2) Multispecies Tests - Qualitative Assessments

Multispecies tests, that is, the use of mixed cultures or communities of microrganisms for a testing protocol, have been used extensively to assess the potential fate and effects of xenobiotics. These tests, support both qualitative (screening) and quantitative assessments (waste assimilatory capacity) (Figure 1). Screening biodegradation tests using microbial communities, for example, commonly follow the disappearence or metabolism of a chemical in samples of water, sediment, soil, or sewage taken directly from the field and incubated in various types of laboratory containers (Howard et al., 1978; Gerike and Fischer, 1979). Insight that is often obtained into modes of biodegradation in actual environmental media could not have been gained from pure culture studies. The ability of sediment associated bacteria to degrade chemicals faster than water-associated bacteria, for example, has come from such studies (Lee and Ryan, 1979; Juengst and Alexander, 1975; Simsiman and Chesters, 1976; Graetz et al., 1970).

Similar enhancements of knowledge (relative to pure culture studies) about the toxic effects of chemicals to microorganisms and the biogeochemical proceeses they mediate have also been obtained in mixed culture studies (Colwell, 1978). Effects of toxic chemicals on heterotrophic activity in aquatic systems (for example, Sayler et al., 1979; Orndorff and Colwell, 1980; Goulder et al., 1980; Albright and Wilson, 1974), on nitrogen cycling in estuaries (Jones and Hood, 1980), and on a variety of microbial activities in soil (Tu, 1980; Atlas, et al., 1978; Anderson, 1978) are representative of the increased amount of environmentally relevant information available from multispecies test as opposed to single-species tests.

The information from a mixed culture test can be evaluated on a relative basis as for pure culture tests. In this case, the environmental risk of a chemical is determined by relative comparisons to chemicals which have a field-documented history of persistence or toxicity in nature. A compound as persistent as Kepone, for example, would have considerable environment risk. However, many chemicals fall into the "grey areas" of degradability and toxicity and thus are much more difficult to evaluate in terms of potential environmental contamination by making relative comparisons of data.

3.3 Multispecies Tests - Quantitative Assessments

Mixed culture test information can also be used for quantitative assessment if a) it involves the determination of rates, routes and extents and b) it can be related to or integrated with some larger picture of the whole (the ecosystem). The necessity for rates and rate constants results from obligatory use of ecosystem mathematical models for predictions of waste assimilatory capacity (Cairns, 1981). Integration into the whole is necessary because mixed culture tests, by the very nature of their pragmatic design and operation, isolate microbial communities from interactions with other communities with other trophic levels and with the physical structure of whole ecosystems. Consequently, although rate information can be derived easily from mixed culture tests, it is basically meaningless by itself until its relationship to the whole or until its coupling to ecosystem function is established and interpreted. It is the authors' opinions that this relationship to the whole is the key to extrapolating laboratory data to the field and the foundation for using microcosms in fate and effect research. As Heath (1979) has stated, an (eco)system is an array of components interacting cooperatively to form a complex unit through which energy, matter, and information may flow. The behavior of the individual components of a system is constrained and coordinated through the network design of the system. Since some of these properities of the system arise not from the components themselves but from the specific set of interactions within the system, it is not possible to characterize a system only from a knowledge of the component parts. Further, only by coupling a holistic investigation of the intact unit to reductionist studies (mixed culture studies) can we hope to understand the natural functions of ecosystems (Heath, 1979). There are many aspects of microbial ecology in which the knowledge and experimentation with a state-of-the-whole is crucial for understanding not only the mechanisms of microbial interactions but also the rates at which they occur.

A microcosm is an excellent method for studying the state-of-the-whole in the laboratory. Figure 1 emphasizes that in order to apply information from multispecies tests to waste assimilation capacity determinations, that is extrapolate laboratory data to the field, studies on the fate and effects of chemicals in microcosms must also be considered. Thus, the role of a microcosm is verification; it basically tells a researcher how to apply information from mixed culture studies to complex real-world situations. It is assumed that the microcosm, as a surrogate of the field, is being used because exposure of the field to a chemical pollutant is unfeasible; a field study would be the ultimate verification if it was practical to implement.

3.4. Application of Mathematical Models

Predictions about the waste assimilative capacitv of an ecosystem, in many cases, will use mathematical models. Models are the only means of evaluating the behavior (fate or effect) of a pollutant when several processes and their controlling variables operate simultaneously and perhaps interact to varying degrees. For example, a good quantitative assessment of the fate of a chemical should collaterally analyze information on the kinetics of each mitagating process (hydrolysis, biodegradation, sorption), on the inputs (time variable, point and non-point) of the chemical into the system, on the physical movement and compartmentalization of the chemical (sorption, sediment transport, mixing, diffusion), and on specific environmental (pH, salinity, dissolved oxygen) and meterological (temperature, runoff) variables associated with a particular ecosystem. The most efficient mechanism for such an analysis is a mathematical description of the total effect that this information will have on the concentration of a chemical through some finite interval of space and time.

Such a description usually takes the form of a large set of coupled nonlinear differential equations that can be generally solved by numerical integration.

This exercise is complicated and expensive. In reaching a compromise between resolution and economy of resources, modelers frequently divide the exercise into two activities: the development of process or analytical models and the development of system or ecological simulation models. The former is always a subset of the latter. The system model concentrates on environmental dynamics or on those forcing functions over which the ecosystem has little control; i. e., runoff from the watershed, insolation, hydrodynamics, sediment scour, and deposition. These are generally environmentally derived parameters that often use the distributional history of a conservative organic chemical (such as Kepone or PCB's) as a calibration source. Each function contributes to the output of the total system by interacting in specific ways with a series of state-variables or components described for that ecosystem. The simulation modeling approach, therefore, is basically a tool for first systematizing knowledge about the complex networks involved in the fate and effects of a pollutant in an ecosystem and second for logically and reproducibly deriving implications about potential environmental hazard or assimilative capacity (Baughman and Burns, 1980). At each stage in the model development, approximations infer some assumption about the real world. Recognition and understanding of the assumptions are the key to evaluating the results of the model.

The assumptions permit the construction of kinetic expressions for the basic processes within ecosystem components which describe, quantitatively, the dynamics, controlling variables, and interplay with various other state variables. For the fate of a chemical, these expressions describe biodegradation, hydrolysis, photolysis, and so forth; for microbial toxicity studies, these expressions would describe population growth, nutrient cycling, mechanisms of biological deficit compensation, and so forth. The development of these kinetic expressions is referred to as process or analytical modeling (Kremer, 1979; Baughman and Burns, 1980). These models couple laboratory-derived process information with the field-derived parameters of the systems simulation model. If information about these processes is insufficient to develop the appropriate kinetic expressions, an experimental program must be implemented to provide that information. It is the opinion of the authors that the success of such a program lies in the quantitative use of microcosms. Microcosms provide a means of testing and verifying the theoretical constructs and assmumptions used in the development of the kinetic expressions under conditions similar to those in the field (Figure 2). Further, any modifications in the assumptions and the theory that are conceived as potential improvements in our predictive capabilities about the fate and effects of pollutants can be tested in a similar manner (Figure 2). As a consequence, the modeler is sufficiently justified to couple the kinetic expressions of the process model to the system simulation model. It should be pointed out however, that the basic information for the kinetic expressions (i.e., rate constants) will come from standard laboratory tests and not from the microcosm (Figure 2). The iterative type process diagramed in Figure 2 is, in the authors' opinion, the most logical and rational way to extrapolate laboratory data to the field.

4. Typical Microcosm Design Features

Since the definition of and approach to using microocsms has been presented, it is important, at this point, to physically describe a "typical" microcosm for those unfamiliar with the array design features commonly employed. Microcosm design is of course closely related to the experimental question being considered, the environmental site being modeled, and the investigator's preference. Consistency in design is only needed in the qualitative use of microcosms.

4.1. Size

Microcosm vessel size generally varies from a volume of several hundred milliliters to one of several hundred liters. For aquatic studies, Pritchard et al. (1979) used a 3.5 cm diameter glass tube containing 200 mℓ of water in contrast to Perez et al. (1977) and Harte et al. (1980) who used glass tanks containing 150 and 200 ℓ, respectively. Quite commonly, 20-gallon aquaria have been used with considerable success (Giddings and Eddlemon, 1979; Sikka and Rice, 1973). Long fiberglass troughs, approximately 30-cm square and 6- to 12-m-long (Hansen and Garton, 1982; McIntire, 1965) have been used to model streams. Terrestrial systems also span a large-size range from 40 cc glass or plastic columns (Draggan, 1977; Jackson et al., 1977) to large glass boxes with volumes in excess of 800 cc (Nash and Beall, 1979; Gillette and Gile, 1976).

The shape and size of the vessel is dictated by level to trophic structure to be studied (incorporating plants, for example, will necessitate larger containers), scaling factors (modeling natural sediment surface area to water volume ratio) and sampling intensity, particularly when sediment or soil is to be removed from the microcosm for analysis. In some studies not directly carried out for microbiological purposes, consistent responses of certain measured parameters could only be obtained with larger microcosms (100-200 ℓ) (Harte et al., 1980; Giddings and Eddleman, 1977). Microbially mediated processes for the most part can be readily studied in smaller microcosms.

4.2. Inoculation

The most important design feature of a microcosm is the inoculum. Ideally, an intact piece of the environment as suggested earlier should be placed in the microcosm vessel as undisturbed as possible. A system concerned with microbial activities only in the water column where interactions with sediment are considered insignificant probably requires no special care in the transfer of the inoculum, except perhaps the inevitable bottle effects. It is questionable whether microcosm studies should even be considered in water column studies. Inocula from sediment and soil are probably best obtained as intact cores. Numerous microbiological studies with intact cores have produced evidence that these cores adequately represent natural conditions in the field (Graetz et al., 1973; Pamatmat, 1971; Cappenburg, 1974; Jones, 1979; Craib, 1965; Van Voris et al. 1980; Twinch and Breen, 1981; Henriksen et al., 1981). The corer itself, depending on the application, can be converted into the microcosm (Pritchard et al., 1979; Van Voris et al., 1980), or the core can be extruded intact into a separate microcosm container. Relatively large cores that can encompass rooted plants and a variety of invertebrate animals are also possible.

Where coring is not possible, container can be filled with natural sediment or soil and returned to the field for acclimation and the recovery of its original undisturbed state. Redox gradients, which are easily disturbed by sampling and may be critical to certain microbial processes, can be restablished in this manner (Flint et al., 1982). The container can then be pulled from the field and transported to the microcosm.

A final alternative is to collect environmental material without concern for natural physical integrity and then allow the material in the microcosm to reorganize itself biologically with the hope that it will hypothetically develop into a miniature, albeit unique, ecosystem. It is assumed that most of the processes and interactions typically seen in the field will be included in the microcosm if given enough incubation time and if the proper inputs are allowed (Lassiter, 1979). Several stream microcosms have been established in this manner by colonizing clean rocks and sand with natural stream water delivered continuously over long periods (McIntire, 1965). The adaptability and functional redundancy in microbial communities makes this approach feasible. Lassiter (1979) suggested that an understanding of the interworkings of these microcosms will aid our understanding of natural processes and their interactions since complexities are comparable. Regardless, the inoculation process is time-consuming, and the criteria necessary to establish the maturity or equilibrium of these ecosystems are not clearly specified. Coring methods appear to be able to provide adequate simulations of the field with a fraction of the incubation time and therefore would tend to supplant this latter innoculation method.

4.3. Mixing

Microcosm studies used to demonstrate the response of bacterial populations to pollutants generally require some type of dispersive action to assure uniform distribution of the pollutant. Aquatic microcosms are generally operated with a completely mixed water column to simpilfy sampling and interpretation of results. Such a mixed state is mechanically quite difficult to obtain, particularly when light resuspendable sediment is present. When replicate microcosms are required, mixing must be equal in all systems. The biodegradation of certain chemicals has been shown in some cases to be closely linked to the presence of sediment (Pritchard et al., 1979; Graetz et al., 1979; Lee and Ryan, 1979) and consequently the degree of mixing will control the degree to which the chemical is physically exposed to the sediments and their associated microorganisms, and the extent of biodegradation.

Mixing is generally accomplished by rotating paddles, propellers, and specially shaped rods. A small motor drive attached to each microcosms is the most efficient. Geared, preset-speed motors are preferred to variable speed motors since, with the latter, constant low speeds are often difficult to maintain. Moderate bubbling with air can provide mixing in small microcosms (Pritchard et al., 1979). Mixing by recirculation through magnetically driven or peristaltic pumps is also very efficient but adds considerable surface area which may lead to additional wall growth and possible adsorption of the pollutant. Application of pollutant to terrestial microcosms is generally accomplished through simulated rain events (Van Voris et al., 1980; Gillette and Gile, 1976; Nash and Beall, 1977). Reasonably uniform distributions have been obtained.

4.4. Other Accessories and Operations

Again, depending on the application, various other microcosm design features must be considered. Temperature is usually controlled by water baths or constant temperture rooms. Lighting is often provided by a bank of 40 W cool-white fluorescent lights that do not present heat problems, and their spectral and intensity qualities are adequate to support plankton and plant growth. Various types of monitoring equipment are often associated with the microcosms providing most commonly continuous measurements of dissolved oxygen, pH, carbon dioxide, and temperature.

Duration of a microcosm experiment can vary from hours to months, depending on the particular application. Often, pretest incubations are carried out to establish base-line information and sustainability in the laboratory. Pollutants can be added to a microcosm in single doses or by continuous dosing. In the latter case, the microcosm can be considered a complex extension of continuous culture systems. Various types of inputs are often required for long-term microcosm experiments; aquatic microcosms frequently receive untreated water as a continual renewal of nutrients and microorganisms.

5. Qualitative Approach For Microcosms

Much in the same way as a mixed culture or multispecies test can be dosed with a chemical and results evaluated on a relative basis, a microcosm, with its additional complexity of maintaining natural physical integrity and higher trophic level interactions in the environmental sample, can be dosed with a variety of chemicals and their results evaluated in a qualitative or relative manner. The actual relationship of the microcosm results to the field is not necessarily the principal point of concern. Instead, reproducibility of both the complexity of the test and the relative responses of the test to a pollutant are the key elements.

5.1. Ecosystem Level Responses

A major assumption in the development of screening microcosms is that through the use of these more complex test systems, new or subtle responses of microbial communities, or ecosystems in general, to a pollutant will be observable. If this is true, then it becomes a question of which parameters or behaviors can be measured. Ideally, measurements of general ecosystem behavior, i.e., the summed results of many processes operating simultaneousy, should be considered. For example, ecosystems can be thought of as microbially based biogeochemical units that have distinct measureable properties (Schindler et al., 1980). These properties may include community respiration, nutrient cycling, oxidation-reduction gradients, organic matter transformation, primary and secondary production. All of those properties are "ecosystem-level" behaviors which reflect the integration of many internal processes and components and their interactions. Ecosystem-level behaviors presumably cannot be inferred or predicted from measurements of isolated components (single species or mixed cultures) in the laboratory (Weiss, 1971). Many of these behaviors are known to reflect a significant degree of homeostasis in an ecosystem, the essence of which is controlled through the tight cycling of certain essential elements by microorganisms and their interactions with higher trophic levels (Fry, 1982). Ecosystem homeostasis is responsible for the maintenance of standing biomass and for the persistence of complex natural systems. It is this interference with the biogeochemical activities of microbial communities that can cause potentially drastic environmental consequences.

Microcosms, as analogs to the field, should also be biogeochemical units showing the same ecosystem-level behavior and homeostasis. As such, a screening program can be set up in which toxic chemicals are administered to a particular microcosm system and the response of the ecosystem-level behavior (fate and effects) measured and compared (to establish risk) on a relative basis, much like the assessment procedures used with bioassay and biodegradability information. Although this approach is still somewhat speculative, some success has been reported (Harris, 1980).

5.2. Screening Microcosms

The original attempt at screening chemicals for biodegradability and bioaccumulation in complex laboratory systems was pioneered by Metcalf and his colleagues (Metcalf *et al*., 1971; Cole *et al*., 1976). These systems were not microcosms by our definition but were synthetic communities established by combining specific laboratory-reared organisms (alga, *Oedogonium*; mosquito larva, *Culex*; water flea, *Daphnia*; snail, *Physa*; fish, *Gambusia*) in an aquarium containing a terrestrial section (sand planted with *Sorghum*) and an aquatic section (reference pond water). The radiolabeled test chemical was applied to the plants (to mimic field application), and the distribution of parent compound and its degradation products through the system determined following the consumption and defecation of the plant material by a saltmarsh catapillar.

By comparing the relative distribution of the chemicals, a "biodegradability index" (the ratio of solvent-unextractable material or polar products found in water or animal tissue to the amount of solvent-extractable material) was created (Metcalf *et al*., 1973). Although the application of the data generated from these screening systems (which includes an impressive number of chemicals) to regulatory decision making has been questioned (Pritchard, 1981; Branson, 1978; Witt and Gillette, 1977; Witherspoon *et al*., 1976), the Metcalf effort is significant as the first attempt to apply "ecosystem complexity" to screening programs. It also stimulated similar experimentation with somewhat more complex synthetic community systems and with emphasis on different types of model communities, namely aquatic (Isensee *et al*., 1973) and terrestrial (Gillette and Gile, 1976; Nash *et al*., 1977; Lichtenstein *et al*., 1978). The synthetic community approach, however, does not consider system-level properties because community interactions are, by the design of the tests, kept at a minimum.

Many of the original microcosm studies of Beyers (1964), Abbott (1966), Cooke (1967), and MacIntire (1965) showed that complex natural communities (biogeochemical units) could be maintained in the laboratory over long periods of time and that these systems, as integrated units, had measurable system-level properties (mainly functional parameters) and homeostatic responses which were not only reproducible but to some extent mimicked similar response measurements in the field (Cooke, 1967; Schindler *et al*., 1980; Odum, 1969).

Relatively little further development of these studies for screening purposes has resulted, particularly as applied to microbial communities. Hammonds (1981) and Harris (1980) have reviewed laboratory studies which potentially could be used for screening microcosms by the U.S. EPA. Two illustrative examples are in order.

Giddings and Eddleman (1978) (also see Harris, 1980) have shown that small ponds can be modeled in microcosms for potential screening purposes. This was accomplished in labortory aquaria by establishing an *Elodea* plant community in sediment samples taken from the ponds. Populations of microorganisms, diatoms, unicellular algae, protozoa, benthic invertebrates, some insect larvae, and the snail, *Physa*, developed in the microcosm into a balanced community within about 6-10 weeks. Thus the microcosm criteria of modeling physical integrity and maintaining couplings with higher trophic levels were considered. Community metabolism [ratio of primary production (P) to respiration (R)] and nutrient cycling were used as the principal ecosystem-level measurements. Distinct similarties in P/R ratios, concentrations of dissolved organic carbon, phosphate, nitrate, and ammonium concentrations and plant and animal community structure between pond and microcosm were observed for an incubation period of 170 days. Coefficients of variation among replicate microcosms were generally below 20%, which is considered acceptable for a screening system (Harris, 1980). This microcosm system can be dosed with a variety of toxic chemicals and their fate (biodegradation and distribution) and effects (on nutrient cycling or organic matter turnover) observed on a relative basis. As Giddings stated (see Hammonds, 1981), "the rankings, not the observed effects, constitute the output of this experimental design." Microcosms have been subjected to stress with arsenic (Giddings and Eddleman, 1978; Harris, 1980), showed significant perturbation in P/R ratios and pH and drastic increases in inorganic nutrients and total organic carbon (Figure 2). The fate of anthracene in these microcosms has also been determined (Giddings *et al*., 1979). These fate and effects studies formed the basis for additional screening of other xenobiotics. Their studies are an excellent example of the screening microcosm approach that can be used to study responses of microbial communities to toxicants.

A soil core microcosm has also been developed for screening chemicals in terrestrial systems (O'Neill et al., 1977; Jackson et al., 1977; Jackson et al., 1979; Draggan, 1977). Again, microbial geochemical processes form the backbone of their toxic effect responses. O'Neill et al. (1977) proposed that the rate at which nutrients are leached from soil could be used as a monitoring point to detect pollutant-induced changes in ecosystem behavior because nutrient flux in terrestrial ecosystems results from the "functional synchrony of autotrophs, heterotrophs, and soil organic matter" (Jackson et al., 1977). Similarily, Van Voris et al. (1980) suggested that CO_2 efflux from the respiration of soil microorganisms could also be used as an ecosystem-level measure of pollutant stress in soil. Support for these suggestions has come from the development and testing of microcosms in which intact soil cores (containing autotrophs) are brought into the laboratory and maintained in plastic columns designed for the continuous monitoring of CO_2 and for the continuous collection of leachable nutrients resulting from simulated rain events. A diagram of the microcosm system is shown in Figure 3. Arsenic and smelter wastes were found to disrupt nutrient cycling concentration of Ca,NO_3-N, NH_4-N, and PO_4-P leached from the microcosm. These changes were a more sensitive measure of effects than those in common population parameters, such as ATP concentrations, bacterial density, and plant growth (O'Neill et al., 1977). An example of this effect is shown in Figure 4.

The characteristics of these microcosms agree well with the tenets suggested for microcosms; in fact use of intact soil cores gave a more sensitive toxic response than homogenized soil (Draggon, 1977; Jackson et al., 1979). Toxicant distribution and extractable nutrient concentrations during treatment of the soil cores were comparable to similar results from dosed field plots (Jackson and Levin, 1979). The functional complexity and ecosystem stability of these soil systems have also been demonstrated at the ecosystem level of organization (Van Voris et al., 1980).

Again, it is important to reemphasize that in the use of these soil core microcosms to screen chemicals for toxic effects, little, if any, information is provided on the mechanisms and specific microbial processes involved in the leaching and respiration phenomena. The concern instead is to rank chemicals for their effects (and fate) in a simple, expedient, and environmentally relevant manner and then draw an arbitrary threshold which will separate the potentially harmful chemicals from those that are harmless.

5.3. Criteria for the Development of Screening Microcosms

The decision regarding when to use a screening microcosm as opposed to mixed cultures studied in a shake flask is often based on nebulous criteria. As a result, microcosms, along with their additional expense and manpower requirements, are often used for screening purposes when in fact there is little justification for their use. Three factors therefore should be considered in the development and use of screening microcosms:

5.3.1 Selection of Most Simple Test

Care should be taken to assure that the conclusion or risk assessments obtained from a screening microcosm cannot be obtained in some simpler way. The mere effort of generating information from the microcosm often overshadows careful evaluation of the results and promotes insensitivity to the question of obtaining the same information from a simpler system. For example, in studies designed to determine effects of microbiota in a microcosm, a toxic response can frequently be attributed to a specific group of microorganisms. By developing a bioassay with this group of microorganisms, the same protection of the ecosystem (worst case analysis) can be realized through a simpler test.

In a similar way, ranking the biodegradability of chemicals with a BOD test is more simple than using a microcosm, particularly if the dependency of persistence on site-specific considerations (which a microcosm might otherwise be designed to handle) is of no interest. Studies in which the fate of a pollutant in a microcosm has been reported are increasing in number, yet it is not clear how the information will be useful in an environmental risk assessment relative to other existing information. For example, Shaw and Hopke (1975) found that the herbicide, diquat, accumulated in the sediments of a microcosm. Nirtanen et al. (1982) reported similar results for DDT. This information, in both cases, could have easily been obtained by using a shake flask and a sediment-water slurry. The extent of sorption to sediments in the microcosms has no bearing on sorption in the field since structural and functional relationships between the microcosm and the field are unknown. The dynamics of diquat and DDT in the microcosms cannot be guaged relative to other chemicals because the fate of additional chemicals have not been studied in microcosms. And finally, the measurement of a pollutant's fate in a complex laboratory system is basically valueless unless it can be shown that the results are significantly different than those generated in simpler tests. In other cases, the abiotic degradation of carbaryl (Liu et al., 1981), the effect of oxygenation conditions on the degradation of PCP (Boyl et al., 1980; Liu et al., 1981), and the effect of input conditions on the degradation of trifluralin (Yockim et al., 1980) are results from complex microcosm experiments which may easily have been obtained from simpler mixed culture flask studies.

To be useful, the screening microcosm must provide insight into phenomena or events which are not readily examined or demonstrated in other previously established mixed culture tests. The soil core microcosm developed by Van Voris *et al*. (1980) and Jackson *et al*. (1977) represents a good example of a unique screening microcosm. No other existing laboratory test provides information on the sensitivity of microbial geochemical processes to toxicants under the complex situations found in natural terrestrial environment. At some future time, however, the sensitive factor(s) that control the nutrient cycling and leaching process may be identified, thus providing the basis for a new bioasay which could then supplant the microcosm approach.

5.3.2 Uniqueness of Results

Part of the concept of water quality criteria and its use of screening data is that maximum acceptable toxicant concentration values are based on the assumption that if the most sensitive species are protected, all other species will also be protected. If the results from a screening microcosm therefore are not unique relative to existing simpler tests, and if they are evaluated in the same qualitative manner as single species or pure culture information, then they must indicate a greater sensitivity to a toxicant (effect at a lower concentration of test chemical) or greater resolution of fate processes than results from other well-established tests. For example, it may be of no consequence that 100 parts per billion (ppb) of a toxicant reduces the metabolism of a microbial community on the surface of aquatic macrophytes (which was previously established as a critical ecosystem function) because free swimming aquatic bacteria are killed at 0.1 ppb. Development of water quality standards to protect most microbially mediated processes would thus use a maximum acceptable toxicant concentration of something below 0.1 ppb. Screening for new effects responses should therefore produce tests which show greater sensitivity to a toxicant than other established screening tools.

5.3.3. Reproducibility and Repeatability

The measurement of microbial community responses in a screening microcosm must possess a degree of reproducibility and repeatability which will allow toxic effects of a chemical to be distinguished from background noise or allow the fate of a chemical to be consistently ranked among a group of previously tested chemicals. The measurement of these responses must show good replication among microcosms within a given experiment and good reproducibility of microcosm results from experiment to experiment. This problem has been specifically addressed in several microcosm studies (Harris, 1980; Harte _et_ _al_., 1980; Stay, 1980; Brockway _et_ _al_., 1979). In general replicate microcosms have coefficients of variation from 10 to 30%, although depending on which parameters are measured, variations can be substantially higher. Functional behaviors of microcosms, such as respiration, production, nutrient cycling, litter decomposition, typically show lower variations among and within microcosm experiments than structural properties such as population composition, species diversity, community biomass (Harris, 1980). Functional parameters also seem to diverge less with long-term incubation (>30 days) of microcosms. Good reproducibility in the distribution of arsenic in water, sediments, and plants (average coefficient of variation was 26%, range was 4 to 45%) has been demonstrated in a pond microcosm system (Giddings and Eddleman, 1977). Nutrient cycling in the same microcosm system showed, in most cases, average coefficients of variation among 3,6, and 12 replicates of less than 20% (Harris, 1980). Concentrations of NO_3, NH_4, PO_4, total P, and total dissolved organic carbon (DOC) in the water columns of twelve microcosm replicates is shown in Figure 5. Brockway et al. (1979) also had similar success in measuring of DOC in pond microcosms. Other variables such as pH, dissolved oxygen, and conductivity also have shown low coefficients of variation (Harris, 1980; Brockway et al., 1979). Many of these functional properties are microbially based and system-level in nature. They could therefore serve as the basis for toxicity testing in a screening microcosm if the effect is greater than the experimental coefficients of variation. Factors such as increased microcosm size (70 to 700 ℓ) and increased pretest laboratory acclimation (0 days to 30 days) (Giddings and Eddleman, 1977; Harris, 1980) also decreased coefficients of variation. Harte et al. (1980), using microcosms which model the pelagic components of lakes, have shown that the decomposition response of microbial communities, as measured by the production of ammonia, to increasing concentrations of added detritus (up to 300% increase over ambient concentrations) was linear and essentially of the same magnitude in each of three replicate microcosms.

Replication and reproducibility of screening microcosms are therefore feasible in relation to responses of microbial communities. However, great care should be taken to examine variability since microcosm design and operational features of the microcosm can greatly affect the results. It is important to further point out that once a particular biological response in a microcosm is established for screening purposes, the same system and its protocols must be utilized for each new chemical tested. Otherwise, the separation of a response to a particular chemical from background noise may be greatly confounded.

6. Quantitative Approach to Using Microcosms For Environmental Fate Analysis

Studies on the fate of the organophosphate pesticide, methyl parathion, in simple sediment-water microcosms (Pritchard *et al*., 1982) illustrate the virtue of this quantitative approach. In these experiments, methyl parathion (20 ppb) was continuously fed into glass vessels containing a 6-cm-deep plug (11 cm diameter) of saltmarsh sediment covered with 400 mℓ seawater; the distribution of the parent compound and its major degradation product, *p*-nitrophenol, followed with time. The water column in these systems was completely mixed but left the sediment-water interface intact. Other studies in our laboratory have shown that functionally this microcosm is representative of a particular part of a local saltmarsh. The concentrations of methyl parathion and *p*-nitrophenol in sterile (2% formalin) and nonsterile microcosms run continuously for 1000 hours are shown in Figure 7. Without any further information, these microcosm results mean very little environmentally; the results could not be used for quantitative predictions of how this saltmarsh could handle a continuous input of this pesticide. For example, hydrodynamic aspects of the saltmarsh have not been considered nor has relative importance of mitigating fate processes been determined. It is not known which conditions in the saltmarsh ecosystem will affect the rates of these processes.

Thus, two additional activities must accompany the microcosm study. A simple process model needs to be developed, and some reductionistic analysis must be undertaken.

6.1. Model Development

Figure 8 shows a conceptual diagram of the potential pathways and distributions of methyl parathion and *p*-nitrophenol in the microcosm. It is a compilation of several assumptions about the fate of this chemical in natural systems. Each reaction (indicated arrows) can ostensibly be described by a kinetic expression. A summary of the expressions used is shown in Table 1.

The expression for biodegradation is particularly interesting, the major assumption being that the rate of biodegradation will be a second-order reaction expressed as some function of a rate constant, the chemical concentration, and the concentration of the catalytic unit. The catalytic unit is presumably bacteria but it could be other factors, such as free enzymes. Bacterial populations or communities will consist of active degraders, some of which will be more active than others. The degraders, under proper conditions, may also change in number due to growth or predation. Paris et al. (1981), on the other hand, have proposed that the biodegradation of pesticides in aquatic environments occurs under nongrowth conditions for the bacteria and that measurements of total colony-forming units on complex media will suffice for the concentration of the catalytic unit. (This assumes that the degraders are always a constant proportion of total population.) They have been able to attribute variations in biodegradation rate of three pesticides in 31 sampling sites to the variations in total colony-forming units. Their proposal is still relatively untested and probably compound-specific but has encouraged research into the problem of measuring the catalytic units and developing kinetic expressions of biodegradation. Lewis and Holm (1982), for example, have shown that under certain conditions, the biodegradation kinetics of methyl parathion cannot be described using measures of total microbial biomass. Bourquin et al. (1981) have reported similar complications. Spain et al. (1981) have shown that for p-nitrophenol degradation, a quantitation of the p-nitrophenol degraders is more appropriate than total biomass.

Once kinetic expressions have been formulated around the necessary assumptions, they can be incorporated into a process model, using the principles of conservation of mass of the general expression:

$$V_1 \frac{dc_t}{dt} = Qc_{in} - Q_{out} + E'_{1,2}(f_{d_2} c_{t_2} - f_{d_1} c_{t_1}) - R_1$$

where V_1 equals the volume of the water column, c is the chemical concentration, Q is the flow through the water column, $E_{1,2}$ is the exchange coefficient between the water column and the interstitial water of the top layer of sediment, f_d is the fraction of the total chemical that is dissolved [i.e., after adsorption to sediment, which is a function of the partition coefficient of the chemical and the organic matter content of the sediment, Karickhoff (1979)] and R equals the summed (assuming no interactions) kinetic expressions in the water (Pritchard _et al_., 1982). Similar expressions account for movement of the chemical from water to the sediment and within defined sediment layers.

6.2. Reductionist Analysis.

With a model in hand, reductionistic analysis is required; a fate study involves the determination of rate constants for each reaction. This is accomplished most simply by sampling water and sediment from the microcosm, placing it in a shake flask, and introducing methyl parathion. Concentrations of the parent compound and _p_-nitrophenol are added with time. Through such studies, sorption rates, equilibrium partitioning, and biotic and abiotic degradation can be differentiated. The rate-limiting variables also can be examined. Abiotic degradation will be pH dependant (Wolff _et al_., 1977); partitioning to sediment will depend on organic content (Karickhoff, 1979). The relationship between biodegradation in the presence or absence of sediment also can be ascertained. Some of the rate constants derived from our flask studies are shown in Table 1. A first-order rate constant was used for biodegradation in water; total biomass was not found to be significantly different between flask studies and microcosm water. A second-order rate constant was used for biodegradation in sediment; catalytic units were equated to sediment mass, assuming an equal distribution of bacteria on each sediment particles.

Figure 9 compares the model's predictions, using the kinetic information from the flask studies, with the actual microcosm data. In both sterile and and nonsterile conditions, a relative good fit of the model to the data was obtained ("good fit" is a nebulous term since it is subjective at this point; differences of less than 4-fold are considered good). From this exercise, several conclusions can be drawn.

1. The second-order description of biodegradation in sediment using sediment mass as quantitative measure of catalytic units proved sufficient to describe the microcosm data. Subsequent studies have shown that a biological "binding" process accounts for the degradation of methyl parathion (Pritchard _et al_., 1982) and probably some of the _p_-nitrophenol (unpublished data), thus corroborating the use of sediment mass as a surrogate for microbial biomass.

2. The kinetic expression for hydrolysis was best described as a second-order rate dependent on hydroxyl ion concentration. Fitting of the model to the data could not have been done without considering that pH in the microcosm in the sediment and at the sediment water interface was 6.8 compared to 7.5 in the water column, thus necessitating two hydrolysis rates. Similar pH differences have also been recorded in the field.

3. Physical diffusion of the pesticide from the water compartment to the sediment compartment was correctly derived from the sterile microcosms. Diffusion into sediment is difficult to estimate since water-mixing and turbulence above the sediment are the dominant forcing function for this

process (Webb and Theodor, 1972). Mixing and turbulence in the field are probably more complicated than in the microcosm; thus, careful calibration of the microcosm with the field must be undertaken (see Sections 11 and 12). Diffusion (molecular and mechanical) therefore could critically govern both fate and toxic exposure of a pollutant in nature.

4. Since the process model for the microcosm treated all fate processes additively, the fit of the model to the microcosm data suggests that this assumption was correct and that there were no subtle interactions or synergisms among fate processes that needed to be included in the model.

5. Finally, the microcosm verified to some extent the kinetic expressions used in model and the environmental significance of the methods used to establish kinetic parameters. To make the predictive jump to the field, the microcosm study is set aside. An ecosystem simulation model (adjusted for the conditions of a salt marsh), such as those proposed by Weigert _et_ _al_., (1975), Clesceri _et_ _al_. (1977), or Burns _et_ _al_. (1982) can then be used to predict the fate of methyl parathion. This model will contain the same "verified" kinetic expressions as above and will take data from the "verified" flask studies, using natural samples from the salt marsh to predict the fate of the pesticide in time and space from a known input in the salt marsh. If the microcosm fulfills our expectations as the verification tool, then our conceptual basis for the extrapolation of laboratory data will be tenable. Our confidence will increase with experience and, where possible, with comparisons to actual field dosing experiments.

6.3. Other Studies

Several other studies have used microcosms in a similar quantitative manner. Falco _et_ _al_. (1977) were able to show that second-order kinetic expressions for biotic and abiotic degradation of malathion were sufficient to predict, through the use of a mathematical model, the fate of this pesticide in a flowing stream microcosm. The microcosm was essentially an elaborate chemostat, having only a water compartment containing microbial and phytoplankton communities and receiving predetermined inlet concentrations of ammonia, nitrate, magnesium, and trace elements. Interestingly, total bacterial numbers were used in determining second-order rate constants in the flask, chemostat and microcosm studies and the close agreement of the rate constants (within a factor of 4) suggest a relationship between bacterial numbers and the degradation rate which is independent of the population composition.

Marshall and Roberts (1971), using a process model to describe adsorption, biotic degradation, photolysis, and fish uptake for the organophosphate pesticide fenitrothion, were able to adequately simulate the change in concentration of the pesticide with time and its eventual distribution in a small farm pond. They concluded that laboratory estimates of the kinetic parameters affecting the fate of fenitrothion, were an accurate reflection of the process dynamics in natural systems (the authors did not experimentally determine the kinetic parameters themselves but rather retreived the data from the literature.). A microcosm study was not necessary in their case because residue analysis information from a field source which had received a dosage of the pesticides was also available from the literature.

The fate of the organophosphate pesticide chlorpyrifos (Dursban) in a pond has been assessed in a similar manner with equal success (Blau and Neely, 1975). Lassiter (1975) showed that simply the construction of a process model, without relating it to the field or to microcosm studies, is sufficient to point out the lack of good kinetic expressions for describing the microbiological and sorptive fate of mercury in aquatic systems. Wolfe et al. (1982) used the EXAMS model (a process model) to predict the fate of hexachlorocyclopentadiene (HEX) in hypothetical simulations of aquatic environments and showed that photolysis and, to a lesser extent, hydrolysis will be the principal fate processes affecting the water and sediment concentrations of this chemical. It would be interesting to see if their conclusions are correct. Since actual dosing of a field site with this toxic chemical would not be allowed, a sediment-water microcosm could be used as a verification of their predictions.

Continual demand for fate information on xenobiotics requires that this quantitative approach to microcosms be exercised frequently. Many other questions about the environmental conditions which affect biodegradation processes could be analyzed quantitatively in microcosms. Many pollutants, for example, are degraded through different pathways and at different rates, depending on the oxygen concentration or redox potential (Shaw and Hopke, 1975; Virtanen et al., 1982; Boyle et al., 1980; Liu et al., 1981; Yockim et al., 1980). Natural environments are typically characterized as having gradients of these factors, and a microcosm is the only means for establishing such conditions in the laboratory and examining their quantitative relationships to biodegradation for the purposes of predicting the chemical's fate in the field. Biodegradation activities controlled by the exudates from plant root systems (Hsu and Bartha, 1979), by litter decomposition processes (Cummins, 1974), and by bioturbation (Hargrave, 1970) are also potential research areas where the quantitative use of microcosms could be applied.

7. Quantitative Approach to Using Microcosms in Toxic Effect Studies

Deleterious effects of pollutants on bacteria have been historically examined in relatively simple experimental systems, primarily shake flask cultures using inocula that are unrepresentative of their original state. Measurements of pollutant effects on the microbial cycling of carbon, nitrogen, sulfur, and phosphorous are some of the most common assays performed in this way. The potential toxic effects of a pollutant on the nitrogen cycle can serve as a good illustration. Toxicologically, the nitrification part of this cycle is a critical effects endpoint since the species diversity of nitrifying bacteria is quite narrow. If these organisms are killed, there is little functional redunancy in the system to accommodate the loss, and recovery may be very slow. This is generally an atypical situation relative to other microbially mediated cycles and heterotrophic processes.

Pure and mixed culture studies on the processes of nitrification, denitrification, and nitrogen fixation have been used numerous times as bioassays for the potential toxic effects of pollutants (e.g., see Atlas *et al.*, 1978). Studies with pesticides (Tu and Miles, 1976; Anderson, 1978), herbicides (Greaves *et al.*, 1976), heavy metals (Bubich and Stotzky, 1980) and other pollutants (Colwell, 1978) demonstrated some type of toxic effect on microbial populations but, by and large, the effects have been bacteriostatic and have occurred at environmentally unrealistic concentrations (10 to 500 mg/ℓ). Very few toxicity studies on the nitrogen cycle have dealt with the response of the microbial communities under conditions where the physical integrity of the natural system is preserved, and the linkage with other trophic state is maintained.

7.1. Role of the Physical Integrity of Sediment in Nitrogen Cycling.

Preserving the physical integrity of a natural system assures that the Eh gradients and dissolved oxygen profiles of sediments and soil are undisturbed. Nitrogen transformations are uniquely linked to the availability of electron acceptors, nitrification and denitrification being basically aerobic and anaerobic processes, respectively. Great confusion currently exists in the literature about exactly how the natural distribution of Eh in soil and sediment quantitatively relates to rates of nitrification and denitrification since microniches apparently permit either process to operate irrespective of the surrounding oxygenation conditions (Painter, 1970; Focht and Verstraete, 1977). Thus a definite need exists to implement microcosm studies with intact environmental samples so that the integrated effect of these microhabitats can be realized and studied relative to data obtained from conventional mixed culture studies. From a toxicological point of view, searching for an inhibitory effect of a pollutant on an isolated component of the nitrogen cycle, i.e., using shake flask cultures to measure nitrification, may represent only a part of the potential impact. Any interference in the biological control of Eh gradients and availability of electron acceptors in soils and sediments by a pollutant can greatly effect the dynamics of the nitrogen cycle. Henriksen et al. (1981), for example, have shown that many nitrifying bacteria in marine sediments, which are normally inactive due to oxygen (or more likely Eh) limitation in a structured sediment situation, become very active in an aerated mixed sediment procedure. They showed that correcting for reaeration in the mixed sediment procedure (Henriksen et al., 1981), or using intact sediment cores (Henriksen, 1980), produced nitrification rates similar to those observed in the field. Charyulu et al. (1980) showed that 2-aminobenzimidzole (a pesticide breakdown product) disrupted the Eh gradient in soil and thereby disturbed the nitrogen cycle through excessive stimulation of nitrogen fixation. Thus, indirect effects of a pollutant on the nitrogen cycle are possible and, most importantly, the effect would go unnoticed in conventional mixed culture tests containing sediment slurries. It also demonstrates the need to understand the kinetics of the nitrogen transformation processes _in nature_ and to know their dependency on certain environmental variables. In the work of Henriksen _et al_. (1980), a quantitative relationship between oxygen concentration and nitrification rate was established and then essentially verified in a microcosm. Other processes in the nitrogen cycle could be treated similarily.

7.2. Role of Higher Trophic Levels in Nitrogen Cycling

Maintaining the linkage between nitrogen flux from sediment and the activities of various trophic levels in the water column is also an important kinetic equilibrium factor in the nitrogen cycle that could potentially be the focal point for disruption by a pollutant. This linkage is based on the supposition that in certain environments (such as coastal marine systems), organic nitrogen input into sediments, through excretions by zooplankton and decomposition of plant, animal, and bacterial remains, is balanced with inorganic nitrogen efflux from sediment and its eventual biological assimilation in the overlying water column. That is, the passage of a significant fraction of organic matter through benthic food chains and its eventual microbial remineralization in sediments controls primary productivity in coastal waters (Nixon, 1981). Seitzinger _et al_. (1980) have shown, using intact sediment-water cores, that Eh conditions permit significant amounts of inorganic nitrogen to be lost from the nitrogen cycle through denitrification in coastal marine sediments (nitrogen fixation is generally insignificant in these environments). Nutrient budget calculations indicate that this loss may account for the low fixed N/P ratios typically observed in coastal marine ecosystem. Nixon (1981) believes this low ratio is the basis for nitrogen-limited primary productivity in these areas. Thus it is not unreasonable to imagine that any pollutant-related perturbation or disturbance to these physiochemical conditions under which nutrient cycling occurs will have a reverberating effects on ecosystem function and productivity.

Burrowing and bioturbation of sediments by various macrofaunal organisms are important in establishing the redox discontinuity layer (Edwards and Rolley, 1965; Fry, 1982) (i.e., the availability of oxygen which would inhibit denitrification), as well as in mixing organic matter down into sediment where active denitrification is occuring (Hylleberg and Henriksen, 1980; MaCall and Fisher, 1980). The balance between nitrification and denitrification is, therefore, probably tightly coupled to the sediment reworking activities of benthic invertebrates (Chatarpaul et al., 1979). Thus, effects of a pollutant on nitrogen cycling may be the result of an indirect response of the microbial populations to physical and biological conditions created by the activities or inactivities of other higher organisms.

To test for these possible perturbations, laboratory systems must maintain the natural Eh profile of sediment because any disturbance to the profile will clearly alter the rate measurements (Jones, 1982). A microcosm with an intact sediment core provides a good laboratory system in which natural sediment organization and oxidation-reduction profiles can be maintained for experimental purposes. This type of microcosm has been applied fruitfully to aquatic (Henriksen, 1981; Seitzinger et al., 1980; Cappenberg, 1974; Pamatmat, 1971) and terrestrial (Van Voris et al., 1980; Jackson et al., 1979) nutrient cycling studies. It offers additional advantages in that the quantitative examination of the couple between water column primary productivity and remineralization processes in sediment can also be undertaken (Nixon et al., 1979). In addition, as stated earlier, the microcosm can be dosed with a toxicant in a manner which is simpler, more controllable, and environmentally safer than a similar dosing in the field.

7.3. Case Study: Effect of Oil Pollution on Nitrogen Cycling

A study to exemplify how a microcosm may be used to reveal a unique toxic response of a microbial community is found in the work of the University of Rhode Island's MERL research group which deals with the effects of crude oil on a coastal marine ecosystem (Elmgren et al., 1980). The researchers successfully modeled Narragansett Bay (Rhode Island) in very large outdoor tanks (13 m^3) (Pilson et al., 1980; Pilson et al., 1979). Tanks (5.5 m high, 1.83 m diameter) contained a box core of sediment (30 cm deep) from Narragansett Bay that received continuous unfiltered bay water (turnover time; 27 days), while exposed to natural light and temperature regimes. Stirring devices were designed to direct turbulent energy onto the sediment to effect resuspension of flocculent material as observed in the bay. The size of these tank systems although originally designed to satisfy a specific sampling regime, greatly restricts experimental manipulation. Further, their cost and technical support requirements cannot be accommodated by most laboratory research programs. However, these systems have been used in some of the most extensively characterized long-term enclosure-type experiments to date, providing detailed descriptions of annual plankton and nutrient cycles, benthic invertebrates, turbulent mixing, and replicability (see Geisey, 1980). They are among the few complex systems to be dosed with a toxicant (Elmgren et al., 1980).

The toxicant study consisted of a chronic (5 months continuous), low-level (range 60 to 350 ppb) dosing with No. 2 fuel oil. Significant effects were observed in both the planktonic and benthic communities (Elmgren et al., 1980). Phytoplankton populations showed a significant increase in biomass and a radically different species composition relative to control tanks. The observed changes were probably a result of decreased grazing pressure caused by a toxic effect to the zooplankton and benthic suspension feeders. Likewise in the sediment, benthic protozoan populations increased dramatically as a result of substantial decreases in predatory macrofaunal populations. In addition, bioturbation activities in the sediment were significantly reduced due to the drop in macrofaunal numbers. Most important from the standpoint of microbial activity was a major suppression in the production of total inorganic nitrogen (NH_4^+, NO_2^- NO_3^-) from the sediments. This is shown in Figure 10. Similar reductions in nitrate generation from beach sand microcosms treated with oil have also been reported (Harty and McLachlan, 1982). The importance of this effect is apparent, considering that the principal growth limiting factor for phytoplankton productivity in Narragansett Bay is controlled by the rate of mineralization of organic nitrogen deposited from the water column into the sediment and the eventual efflux of nitrogen from sediment (Nixon, 1981).

Elmgren et al. (1980) demonstrated that effects observed in their study could not have been predicted from knowledge of the biology and susceptibility to oil of the individual species. However, require further dissection and analysis of the test results (state-of-the-whole) are required to provide greater comprehension of the interactions involved and to the develop a predictive model to forecast similar toxic effects in other environments. For the sake of our illustration, assume that similar effects on the nitrogen cycle can be demonstrated in small laboratory microcosms. This is probably a valid assumption because Adler et al. and Nixon et al. (1979) have indicated that much of the same ecosystem structure and function in the MERL tanks (coupling between pelagic and benthic components) can be modeled in 150-liter microcosms.

7.4 Model Development

Quantitative use of a microcosm to study the toxic effects of a pollutant can be performed, in theory, by first establishing a state-of-the-whole in the microcosm and then measuring the effect of a pollutant on that state. Extrapolation of this potential effect to the field necessitates a series of research steps similar to those described for fate studies.

A conceptual process model for the microcosm results must be developed to clarify the kinetic relationships among system components. The model should reflect current knowledge and assumptions about the processes suspected to operate in the system of interest, particularly those that are possibly sensitive to the pollutant. All of the kinetic expressions for the system processes, pathways, and component interactions necessary to provide the required outcome must be represented.

Rate information for each process involved must be obtained either by using literature or by experimentally determined values. Incorporation of this information into the process model will yield predictions (such as nutrients flux from sediment) which can be compared with the undosed microcosms. If the model is unable to predict the microcosm results within some degree of error, then the iterative process outlined in Figure 6 can be used to modify the kinetic expressions for the model.

Once the model has been adjusted to provide reasonable predictions, various rate functions can be adjusted to simulate positive and negative responses to a pollutant input and the end result compared with the results from the dosed microcosm. The implicted processes can then be experimentally tested, assuming the laboratory methods for their isolation and study have been worked out, for the sensitivity to the pollutant. Dose response relationships can subsequently be established.

In the oil-dosing experiment described above, it is reasonable to hypothesize that the reduction in total nitrate efflux from sediment could have been caused by a change in the redox profile of the sediment resulting from a toxic effect of the oil on bioturbating macrofauna. Bacterial populations themselves are generally not chronically poisoned by oil pollutants (Colwell, 1978). A change in the redox profile, however, could dramatically increase denitrification rates since it is already believed that 5 to 20% of the nitrogen remineralized in these sediments is lost as N_2 and N_2O (Seitzinger et al., 1980). Studies have shown that pollutants will affect the bioturbation activities of benthic invertebrates. Rubinstein (1979), for example, has shown that Kepone at sublethal concentrations inhibits sediment reworking activities of the lugworm (Arenicola cristata); this was observed in a bioassay system in which the extent of detrital loss from the surface of sand (i.e., incorporated into the animal burrow) was measured photographically. A linear dose-dependency was also observed.

7.5. Model Development

The hypothesis concerning increased denitrification due to oil pollution could be tested with a process model describing the relationship among biological sediment reworking, control of Eh/dissolved oxygen profiles, and control of nitrogen remineralization. To our knowledge, this has not been attempted although available literature seems to indicate that the task is feasible. A possible, highly simplified conceptual model of this role of bioturbation in nitrogen cycling is shown in Figure 11. Included is a model of the nitrogen cycle developed by Lassiter (1975) and the two-layer model reported by Vanderborght and Billen (1975). Each arrow represents a process or group of processes for which some kinetic expression and some method of rate measurement are needed. Most of these expressions take the form of biomass dependent first-order reactions:

$$k_2 = [b]e^{-k_1 t}$$

where k_2 is the apparent rate constant, k_1 is the exponential substrate dependence term, and b is the concentration of biomass; for example, bacterial or invertebrate population size. However, a number of mechanistic models describing nitrogen transformations in soil have assumed first-order (with respect to substrate concentrations) reactions (Metiran and Tanji, 1974). Others have factored in microbial growth using the Monod equation. Bazin et al. (1976) have presented an excellent review of those nitrogen transformation models developed for soil systems. In Lassiter's model, oxygen concentrations are used to switch the nitrification and denitrification processes on and off, simulating aerobic and anaerobic conditions. Each major dynamic variable (see Figure 11) can be expressed as a sum of all the controlling parameters. For example, the parameters used to describe the change in concentration of ammonia are shown in Table 2.

Values for each parameter can be asessed by either application of literature values or by appropriate experimentation. Since the literature on the microbiology of the nitrogen cycle is extensive (Focht and Verstraete, 1977), much of the process information can come from that source. Hopkinson and Day (1977) have listed many of the rates typical for nitrogen cycle processes in a salt marsh. In other cases experimentation will be required. The work of Henrikson et al. (1981) that showed the relation of oxygen concentration to the rate nitrification has been cited. Jahnke et al (1982). successfully modeled the quantitative dependence of denitrificaiton on sediment respiration, using profiles of pore water nutrients. Hansen et al. (1981) have demonstrated the relationship of nitrification rates to sediment depth, Eh, and season. Fry (1982) has discussed the methods for relating bioturbation to reaeration of sediments. Hyllebert and Henriksen (1980) and Henriksen *et al*. (1980) have quantitated the effects of bioturbation on nitrification rates. Jones (1979) has demonstrated a quantitative relationship between Eh values and denitrification rates (measured as nitrate reductase). Extensive measurements of redox-potential in the sediments of the microcosms must also be carried out both with and without the oil exposure. This can be accomplished either with the dehydrogenase assay technique (Pamatmat and Bhagwat, 1973; Vosjan *et al*., 1977) or directly with platinum electrodes (Howes *et al*., 1981).

Finally, information will also be needed on measurements of sediment reworking activities. They can be estimated indirectly from measurements of respiration in sediment (McCall and Fisher, 1980) as long as data are available on the denisty, physical movement, and burrow configuration of the principle bioturbating invertebrate. This information is available in the literature or can be obtained experimentally, using existing measurement methods.

With this information, the validity of the model can be iteratively tested by referencing the model outputs with results from the microcosm and readjusting the model (or the kinetic descriptions of the processes) until adequate predictions of the microcosm results are obtained (see Figure 2). Once confidence in the predictiveness of the process model is acquired, it can be integrated with an ecosystems simulation process model to account for spatial and temporal parameters (geomorphology, water hydrodynamics, reaeration by turbulent flow, deposition rates of organic nitrogen, variations in Eh gradients from site to site, steady state, and time variable concentrations of inorganic nitrogen in the water column) and couplings between nutrient efflux from sediment with phytoplankton and zooplankton population dynamics which cannot be observed or accomodated in small microcosms. The possibility then exists of demonstrating that the impact of small change in the activity of a microbial community in response to a pollutant (depressed nitrogen efflux from sediment) can be magnified significantly after being coupled to other trophic activities of the ecosystem (primary producivity). Examples of ecosystem simulation models have been described by Clesceri et al. (1977), Hopinkinson and Day (1977), and Nixon and Kremer (1977). It should be apparent that the rate measurements of the processes in the nitrogen cycle and a quantitative knowledge of the rate controlling parameters, including the effect of a pollutant, are results that are extrapolated to the field, not the microcosm results.

The importance of using models as interpretative tools and of using microcosms as representations of the state-of-the-whole in the study of activities of microbial communities cannot be overemphasized. Unfortunately, very few process modeling efforts have been coupled with a microcosm study in the area of microbial nitrogen cycling, or any other cycles for that matter. Even in the application of models to nitrogen transformations in soil, Bazin et al. (1976) have pointed out the need to produce more complete laboratory simulations of nitrogen turnover in natural systems so that more of the "black box elements" can be replaced with analytical descriptions of relevant processes.

7.6. Additional Examples

Model development may suggest a need to experimentally investigate a new process or interrelationship. For example, in working with nitrogen cycling in estuarine salt marshes, nitrogen fixation should probably be considered as a major factor in regulating the nitrogen budget. Mann (1979) showed that measurements of N_2 fixation in bell jars placed over Spartina and other salt marsh plants in the field yielded rates considerably faster than those projected from a separate study of cyanobacteria taken from the mud surface (amounting for only 20% of the total N_2 fixation). The bell jar experiments in this case provided the state-of-the-whole since the field site was physically accessible. If, however, the effects of a pollutant are to be investigated, a microcosm may be required (it is feasible to take intact sediment cores with associated macrophytes for this purpose.). Laboratories studies by Mann (1979) indicated that N_2 fixing bacteria associated with the isolated root parts of Spartina (two bacterial types have been isolated, a facultative anaerobe and a microaerophile) were quite sensitive to oxygen concentrations (5 to 9% was optimal) and to the supply of dissolved nitrogen compounds in the surrounding milieu (figure 12). This explained the observed higher rate of N_2 fixation associated with the plants along the edges of the marsh that were exposed through tidal action to seawater containing dissolved nitrogen compounds.

From a process modeling standpoint, kinetic rate expressions could be developed for all sources of N_2-fixation and for the environmental factors controlling each source and then integrated into a model. The model can be checked to verify if it predicts the N_2-fixation rates observed in situ or in a microcosm. By examining microocsm (or field) results and considering the regulation of microbial activity in relation to the physical integrity of natural systems and the interaction with higher trophic forms, a new site for the possible adverse effects of a toxicant can be realized. It may be considerably more sensitive than the direct tests on pure cultures of cyanobacteria.

Another excellent example of applying a process model to a set of microcosm experiments is the work of McIntire on the dynamics of lotic periphyton in laboratory streams (McIntire, 1978; 1966; 1965; 1964; McIntire et al., 1975). His first approach was to operate the stream microcosms in the laboratory and thoroughly examine the periphyton community structure and population dynamics as a function of light intensity and current velocity. This was basically an exercise in manipulating the state-of-the-whole (see section 10). He then attempted to document the major controlling variables affecting the processes of primary production, community respiration, introduction of organic and inorganic materials from outside the system, export of organic and inorganic materials, and the grazing activities of aquatic animals. A kinetic expression was developed for each process based on the literature and the data from a variety of field and laboratory studies (McIntire et al., 1975; McIntire, 1978). Data describing the system's variables, such as temperature, light intensity, current velocity, nutrient concentration, silt loading, and organism biomass, were incorporated into these expressions where appropriate. By then combining these mathematical descriptions into a process model and running the model under steady-state conditions, predictions of periphyton dynamics were compared with microcosm results as a means of validating the model.

Toxicological studies can now be applied to this analytical framework. For example, the effect of pollutant on a microbial community responsible for the mineralization of organically bound nitrogen or phosphorous from leaf litter can be translated to an effect on periphyton dynamics in stream ecosystems. Similarly, the toxicity of a pollutant to grazers (snails, in this case) can be examined for "domino" effects on microbial mineralization rates and ultimately periphyton dynamics. Interpretation and field extrapolation of the results from stream microcosms exposed to a pesticide (Hansen and Garton,

1982) and to fuel oil (Bott and Rogenmuser, 1978) could probably be enhanced by applying the McIntire model.

Several other experimental situations have been reported which might allow a quantitative demonstration of the toxic effects of a pollutant to a microbial population. Fry and Ramsey (1976) reported on the indirect changes in heterotrophic activity of microbial epiphytes on two aquatic macrophytes, Elodea canadensis and Chara vulgaris, following treatment of the plants with the herbicide, paraquat. The kinetic parameter, K + Sn, where K = the half saturation constant and Sn = the natural substrate concentration, for glucose mineralization was normally quite high (36 to 150 μg/ℓ) for epiphytes on Elodea, suggesting that healthy plants secrete dissolved materials that are then available to the bacteria. Killing the Elodea with paraquat significantly increased the K + Sn and Vmax (maximum glucose uptake rate) of the epiphytes, which was not the case with the epiphytes on the paraquat-resistent Chara. The increase in kinetic parameters was not the result of increases in population size but rather the physiological activity of each cell apparently changed. This coupling of microbial community responses with higher trophic level activities is another example of potential indirect effects of a toxicant to bacteria. It is a relationship which requires a natural physical association which could be accommodated in a microcosm.

The availability of methyl mercury in sediment is controlled by several processes operating simultaneously at a rate dependent on environmental parameters closely tied to physical structure of sediment beds and to physical interactions with other trophic activities. Since mercury could not be used in the field, microcosm studies are required. Bacterial populations in sediment can convert elemental mercury to the mercuric ion form (Nelson and Colwell, 1975) and can then convert this product to methyl mercury (Jensen and Jernelov, 1969). The methyl mercury can subsequently be degraded by other bacterial populations (Billen et al., 1974) which appear to adaptively increase their mineralization capacity with exposure to increasingly larger concentrations of methyl mercury (Billen et al., 1974). This set of processes is affected by anaerobic conditions, the amount of organic matter (Olsen and Cooper, 1976), perhaps by salinity (Blum and Bartha, 1980), and by the presence of invertebrates which can rework the sediment (Jernelov, 1978).

Without a process model and some complex natural system to use as an integrating tools, it is very difficult to make quantitative predictions regarding the net rate of methyl mercury accumulation in an ecosystem. Some effort has been made to develop kinetic expressions for each of the processes affecting the fate of elemental mercury in natural systems (Bisogni and Lawrence, 1975), and results from a microcosm study have been reported (Titus et al., 1980). The latter study provides interesting information on distribution and partitioning of mercury between sediment and water. Without the use of a process model, however, the results from the microcosm have little predictive value.

8. Interaction Between Microcosms and Simpler Systems

The holistic approach to studying a microbial community in the laboratory, when combined with the classic reductionist approach of studying small pieces of the whole (pure or mixed culture studies for example), is a potentially productive way to increase understanding of microbial ecology, particularly as it relates to pollutant-mediated responses. Part of the beauty of this combination is that one approach can be graded into the other through a series of scaled successions, theoretically by either a "scale-up" to or a "scale-down" from a complex natural situation.

In the "scale-up," specific types of pure and mixed cultures can be studied together in various combinations, and the end result compared with the a complex situation (state-of-the-whole). The intent is not to reproduce the whole exactly but instead to compare the relative contribution of each pure or mixed culture component. This has been tried several times in microbial ecology with moderate success, although in many cases a state-of-the-whole or microcosm study is not included. A good example is the work of Coleman and his colleagues (Coleman et al., 1978a, 1978b; Herzberg et al., 1978; Anderson et al., 1978; Cole et al., 1978), in which a series of factorially designed experiments were employed to investigate the effects of trophic interactions on the mineralization of organically immobolized carbon, nitrogen, and phosphorous in soil. The trophic interactions were modeled as a simple food web consisting of various combinations of plant exudate and pure cultures of bacteria, amoebae, and nematodes inoculated into sterilized soil. The researchers established that once phosphorous was immobilized by bacteria, it was rapidly remineralized by the saprophagic grazing of the amoebae and subsequently released as inorganic phosphorous. Although these results are informative, their environmental validity could be greatly enhanced by a comparison with the complex state of a microcosm. The microcosm could be an intact soil core in which the leaching of inorganic phosphorous after exposure of the core to plant exudate could be measured. Since the activity per organism had been established separately, the biomass of the bacteria, ameobae, and nematodes in the core could be determined and monitored with time. A simple model could then be used to see how well the individual pieces predicted the whole. The suitability of this particular example in measuring response to the addition of pollutants is obvious. A classic study of leaf litter decomposition and mineral cycling in forest ecosystems was carried out by Witkamp and colleagues (Patten and Witkamp, 1967; Witkamp, 1976; Witkamp and Ausmus, 1975), using the same type of factorial experimentation with a variety of simple components (leaf litter, microflora, millipedes, snails, soil). Again, the synthesis of various degrees of complexity was never compared with the natural complexity in the field or in a microcosm.

The "scale-down" situation involves both manipulation of whole and experimentation with isolated microbial communities or subsystems that are derived by disassemblying the whole. This is likely to be a more productive situation than the "scale-up" exercise. The usefulness of the microcosm, in this case, is to isolate and illustrate important mechanisms that control the real system. Our studies on the fate of p-cresol illustrate how the complex results from a microcosm can be manipulated and disassembled into subsystems to provide quantitative information on the factors controlling the fate of a chemical in an aquatic system and provide input data for a mathematical model. In conjunction with the dosing of several man-made stream channels with the toxicant p-cresol (Cooper et al., 1982), we performed laboratory studies on the fate of p-cresol in order to see how accurately we could predict its fate in the field streams. A microcosm study, involving the fate of p-cresol, was therefore conducted. The microcosm design (Figure 13) used a tray technique (36 cm diam, 10 cm high) to transport intact portions of the riffle area and pool area in the streams to the microcosms. Plants associated with these areas were included in the trays. A recirculation device provided complete mixing of the water column (80 liters) and produced turbulence similar to that in the stream. The stream channel was actually a closed recirculating loop and consequently the microcosms, as a riffle-pool pair, were maintained as closed systems. Since the stream was autotrophically based, diurnal dissolved oxygen profiles were used to calibrate the microcosm with the field. Invertebrate populations on the rocks and sediment were also used as calibration points. In both cases a good simulation of the stream was obtained by the microcosms.

The microcosms (riffle-pool) were spiked with 8 ppm p-cresol; the disappearance of the parent compound followed high pressure liquid chromatographic analysis. The result (Figure 14) is representative of the state-of-the-whole; the rate of disappearence of p-cresol is controlled by an unknown number of factors whose integration produces the microcosm result. No loss of p-cresol occurred in azoic microcosms.

It is important to point out that this microcosm result has little predictive value of it own. The microcosm is not a duplicate of the stream, and the results have no quantitative bearing on the rate of disappearence of p-cresol in the stream channel. Therefore, only a qualitative judgment about its fate in the stream can be made. However, a quantitative extrapolation of the microcosm result to the field is possible if the controlling variables which affect the compound's fate are demonstrated; i.e. kinetic information about the processes involved, specifically rate constants.

To more clearly understand the factors controlling the fate of p-cresol, the rate of degradation of p-cresol in microcosms containing only riffles and only pools was compared. It can be seen in Figure 15 that the all-pool microcosms degraded p-cresol slower than the all-riffle microcosms. Degradation rate in the pool-riffle microcosm was among the individuals. The manipulation indicated that stream riffle areas were quite important in the initial biodegradation of p-cresol. Respiking of the microcosms produced similar degradation rates in all systems (Figure 15), indicating that the biodegradation contribution from the riffles is important in initiating degradation of p-cresol.

Disassembling the state-of-the-whole into subsystems (that is, microbial communities) to obtain more quantitative information was straightforward. Mixed microbial populations associated with water, sediment, rock surfaces, and plant surfacs were separated from the microcosm and studied in shake flasks to determine their p-cresol biodegradation potential (Pritchard et al., 1982). Figure 16 shows the degradation rates of p-cresol in shake flasks containing components from the stream. Bacteria associated with rock surfaces were the most active degraders. These subsystem or mixed culture experiments provided degradation rates per unit mass or rate constants, i.e., rates per liter of water, rates per rock, rates per gram of sediment. By knowing the approximate mass of each component in the stream channel, a prediction of the rate of disappearence of p-cresol during dosing can be made using a mathematical model (for example, the EXAMS model, Burns et al., 1982). Criteria for integrating the rate information come from the microcosms. Adaptation to faster degradation rates appeared to have been coupled to some factor associated with rocks or sediment since adaptation in water-only shake flasks took much longer than in the microcosms (data not shown).

Another interesting form of the "scale-down" considerations is suggested in the adaptation studies of Spain _et_ _al_. (1981). A typical adaptation of microbial populations to greater biodegradation activity of p-nitrophenol (PNP) in a small microcosm using a sediment-water core from fresh and saltwater environments is shown in Figure 17. Estuarine environments in most cases have shown no adaptation within the same time frame needed to see adaptation in freshwater environments. In an attempt to better understand this lack of adaptation in estuarine environments, several potential manipulations could be undertaken. For example, Spain (personal communication) has isolated a PNP degrader from an estuarine site, using a selective enrichment technique. This presents the possibility of seeding an estuarine microcosm with the isolate to see if adaptation can be provoked. If adaptation occurs, it suggests that environmental conditions in the microcosm do not preclude this event; rather, it would appear to be a deficiency in the indigenous microflora (very low numbers of PNP degraders which might grow slowly). If adaptation did not occur, it would suggest that the environmental conditions of an estuarine environment, relative to its freshwater counterpart, were not conducive to PNP degradation. Either way, this manipulation of a microcosm specifically directs the research toward determining why estuarine environments cannot degrade this pollutant.

9. Field Calibration of Microcosms

Simulations of ecosystem components in microcosms requires some verification, generally by making comparisons to the field. Since exact simulation is unlikely, it is important to check various parameters and behaviors of a microcosm to determine if they agree, within certain acceptable limits, with similar parameters and behaviors measured in the field. If the behavior of the microcosm falls outside these limits, then it is theoretically possible to change the microcosm design or operating conditions to bring the behavior within the acceptable limits. This exercise was referred to as the field calibration of microcosms in our studies (Pritchard, 1981). "Acceptable limits" are subjective evaluations related to the investigator's previous experience and to consensus judgments based on the literature. A calibrated microcosm should infer that a degree of similarity (or dissimilarity) between the laboratory and field has been established and can be factored into or integrated with the interpretation of microcosm results. Processes and behaviors in a microcosm may differ considerably in magnitude but should show the same trends as the field.

9.1. Functional Attributes.

There have been some efforts to establish the parameters and behaviors of an ecosystem that are most suitable as indicators of differences between microcosm and field (Perez et al; 1977; Harris, 1980; Jassby et al., 1977). Functional attributes of communities in an ecosystem, as opposed to structural attributes, are most frequently utilized for this purpose. Measurements of functional responses, although reflective of general ecosystem behavior, are quite often mechanistically based on the activities of microbial communities. They are typically community responses which theoretically can be attributed to a variety of ecosystem community structures and trophic interactions. In other words, they are measures of system behavior which empirical studies shown to be indicative of ecosystem well-being and comparability.

Primary productivity and respiration are key functional parameters of most aquatic and terrestrial ecosystems. Substantial evidence now exists for aquatic systems that support the concept that the ratio of production (P) to respiration (R) is an excellent measure of system behavior and is consistent, sensitive to man-made perterbations, and easily compared in both laboratory and field situations (Giddings and Eddleman, 1978; Beyers, 1964; Cook, 1974). Odum (1969) suggested that a P/R ratio of one can be attributed to mature ecosystems; this has been observed in both microcosms and natural systems. A reduction in the ratio signifies a negative energy balance; the particular system does not maintain itself and will continue to show a decrease in P/R ratio if no allochthonous or autochthonous energy sources are provided. A measurement of P/R ratio in an aquatic microcosm will consequently provide some indication of its self-sustaining characteristics relative to the field. It is thus reasonable to assume that a microcosm which tracks the P/R ratio of the field is likely to track other natural microbial processes in the field as well.

Nutrient cycling is, from a microbial community standpoint, probably the best field calibration checkpoint available. Dissolved inorganic nutrient concentrations in the water column or interstitial water of many aquatic systems are typically quite low due to the close coupling of remineralizations rates, plant uptake rates, and physical exchange rates between water and sediment. A microcosm which shows higher water concentrations relative to the field indicates that this couple may not be functioning realistically and that the laboratory system is possibly not self-sustaining. The extent that the response of microbial populations to a pollutant will be affected is uncertain, but generally it is believed that a good microcosm simulation should at least mimic nutrient cycling events in the field. Examples of how field calibration with nutrient cycling can be assessed are found in the nitrogen and sulfur cycling studies of Henriksen (1980) and Cappenburg (1974), respectively.

Biogeochemical cycles in soil and sediment are also closely linked to proton and electron activity or redox potential (Eh). Eh is an integrated measure of many chemical processes and redox reactions. Disturbing the redox gradient, as discussed previously, can lead to significant changes in metabolic rates of carbon turnover and mineralization by certain microbial communities. Consequently, a microcosm which does not model natural redox conditions is likely to provide a poor field simulation. Redox potential can be measured either by metallic probes (Schindler et al., 1980), by oxygen uptake (Edberg and Hofsten, 1973; Pamatmat, 1971), or by assays for dehydrogenase or electron transport system (ETS) activity (Dlanczuk-Neyman and Vosjan, 1977). In each case, good correlation between the microcosm (usually an intact core) and the field has been observed. Figure 18 shows how a tidal flat microcosm was field calibrated, using vertical distribution measurements of ETS activity, oxygen uptake, and sulfide concentrations (Vosjan and Olanczuk-Neyman, 1977). Similar measurements could also be made in terrestrial systems. The measurement of dissolved organic carbon (DOC) concentrations resulting from secretions by living organisms and partial decomposition of dead organisms is another potential field calibration measurement. Transformation of organic carbon and the subsequent release of nutrients are the major growth controlling factor for the autotrophic community. As such, DOC would be expected to vary as a function of both autotrophic and heterotrophic activity. Burney et al. (1981) compared the flux of dissolved organic carbon and polysaccaride carbon (CHO) in a saltmarsh and a large outdoor tank and found not only significant differences between the tank and the field but a strong relationship between bacterial activity and the DOC or CHO levels. They suggested that bacteria may respond rapidly to and control the concentrations of polysaccaride carbon and perhaps other types of dissolved organic carbon. Similar conclusions were reached by Larsson and Hadstrom (1979), Wiebe and Smith (1977), and Straskraboua and Fuksa (1982). Simulation of DOC turnover in a microcosm may therefore be a very sensitive field calibration method.

Concentrations of DOC also reflect the eutrophic condition of a natural system (Wangersky, 1978). In light of the fact that a microcosm will contain excessive wall surface area for algal growth, DOC may be considerably elevated over natural conditions. This could have a significant effect on metabolic turnover by microbial communities, and consequently the fate of a pollutant. A misleading evaluation of risk assessment might therefore result if DOC and the associated bacterial activities are not calibrated with the field.

The measurement of CO_2 evolution rates from soil is a tested and reliable technique for assessing general biological activity. It is also a sensitive measurement which is often affected by sampling procedures and the subsequent incubation conditions. As with the other field calibration techniques, CO_2 evolution is an integrative measure that does not distinguish the relative contribution of the different living components in the sample, i.e., microorganisms, plant roots, and invertebrate animals. Ausmus et al. (1978) have shown the usefulness of this technique in comparing the litter composition behavior of a "treecosm" with the field under similar regimes of heavy metal exposure.

9.2. Structural Attributes

Structural features of a microbial community may also provide information useful in calibrating a microcosm with the field. Although it is a delicate and time consuming undertaking to describe a bacterial community according to certain species diversity indices, it is feasible under certain circumstances. Taxonomy of bacteria, because of poor morphological differentiation, commonly relies on the physiological, metabolic, or biochemical characteristics of bacteria to determine species composition and diversity. Troussellier and Legendre (1981) have suggested a unique "functional evenness index" which is based directly on the results of the API 208 testing method. The availability of this testing method would make the application of an evenness index feasible in calibrating a microcosm. Hauxhurst _et al_. (1981) have developed indices of physiological tolerance and nutritional utilization which could be used in a microcosm study to be certain that the natural diversity of the microbial communities was maintained. The nutritional utilization indices in this study were based on the qualitative parameter of presence or absence of growth on a large variety of single carbon source media. Similar indices could probably be established for the quantitative utilization of a substrate, using standard ^{14}C heterotrophic activity measurements. Goulder _et al_. (1979, 1980), for example, have shown that the Vmax/bacterium for glucose mineralization in estuarine water samples was negatively correlated with industrial pollution, particularly, stresses due to sewage and copper. However, if the containment of a microbial population in a microcosm is considered stressful in itself, the resulting heterotrophic indice could be a good indication of field simulation.

White and colleagues (1979) have pioneered a biochemical approach of assessing prokaryotic and eukaryotic biomass and community structure in natural systems in which a qualitative analysis of lipid and fatty acid compositions in a sample is used. This approach is an excellent means of comparing sediment microbial community structure (Bobbie and White, 1980) in natural ecosystems and microcosms. For example, most bacteria contain diacylphospholipids. Actinomycetes, corynebacteria, and most eukaryotic protists contain a high proportion of ether-, vinyl ether-, and amide-phospholipids. Anaerobic bacteria exclusively contain plasmalogens. Short chain (13 to 21 carbons) iso- and anteisobranched fatty acids are typical of gram positive bacteria, and cyclopropane fatty acids are largely associated with gram negative bacteria (White _et al_., 1978). If the relative composition of these compounds in an environmental sample is unique, then tracking of the microcosm biochemically with field is possible. Various kinds of drilling mud components have been shown to alter the lipid and fatty acid composition of sand colonized by the biota from flowing seawater (Smith _et al_., 1982).

There are many other potential field calibration measures available. Almost any general parameter of bacterial community structure and function which is sensitive to various kinds of stresses is applicable to microcosm design and operation because it can be assumed that a microcosm behavior which deviates from that observed in the field is for all practical purposes stressed. Adenylate energy charge (Chapman and Atkinson, 1977), a variety of enzymatic activities (Griffiths _et al_., 1982), especially chitinase activity (Portier and Meyers, 1981), the microbial composition of the sea surface microlayer (Hardy and Valett, 1981), and of course a whole range of heterotrophic activity measurements are all possible field calibration measures.

9.3. Fate Studies

A final type of field calibration approach is to examine the handful of cases where the fate of a particular chemical in a microcosm has been compared with a controlled field plot. Several studies indicate differences in biodegradation rates between lab and field; such examples could be examined further to determine what factors in microcosm design or operation were responsible for these differences. Sikka and Rice (1977), for example, compared the fate of the herbicide endothall in a farm pond and a laboratory aquarium microcosm. Their results are shown in Figure 19. Overall, the same pattern of distribution (partitioning to hydrosoil) and disappearence of parent compound was the same, but the magnitude of these processes were considerably different. Endothall accumulation in the microcosm sediments was approximately one-half that found in pond sediments; the highest concentration of herbicide in sediment occurred much earlier (a difference of 20 days) in the microcosm and the loss rate of parent compound from the sediment after peak concentration was much more rapid in the pond. Degradation in the water column was much faster in the microcosm due primarily to a shorter lag in an apparent adaptation to greater biodegradation rates. The reduced lag may have resulted becasue the microcosms were established originally with pond water and sediment was taken from a control pond that was different from the dosed pond. The latter had been previously (12 months earlier) exposed to endothall (Sikka and Rice, 1973). The other differences might be attributable to approximately a nine-fold difference in sediment surface-area to water-volume ratio between pond and microcosm and possibly slow mixing in the pond. The potential for calibrating the microcosm with the pond is apparent.

Tsushimoto _et_ _al_. (1982) compared the fate of TCDD in an outdoor pond (manmade) and in 16-cm-diameter glass bottle microcosms. Data from each test system were roughfully comparable for short-term distribution behavior. However, a large die-off of the pond weeds, _Elodea_ and _Ceratophyllon_, in the outdoor pond caused a significant change in the overall physical distribution of the TCDD probably because of large increases in sorptive surface competing with similar surfaces on the sediment. It is not clear whether a similar shift would have occurred in the microcosm study since a pond weed die off did not occur with the shorter incubation period. Significant distributional differences may have resulted if plant die off in the microcosm had occurred. The relative proportion of sediment, water, and plant components in the microcosm was not calibrated with the pond.

A related microcosm study involving the effect of plant material on the fate of diquat also indicates the need for field calibration. Simsiman and Chesters (1976), using flasks containing static sediment-water components as simulated lake impoundments, showed a significant depression in diquat degradation because of oxygen depletion in the system caused by the death and decay of plant material. Their suggestion that this may be a potential result in the field is very tenuous, considering the disproportate ratios of water volume to plant mass between the flask and any field situation.

Kloskowski _et_ _al_. (1981) have reported a study comparing the degradation (including conversion to bound residues and carbon dioxide) of 11 different chemicals in a soil-plant (summer barley) microcosm to that occurring in an outdoor experimental area. Relative persistence of the chemicals was similar in both lab and field tests but the magnitude of degradation in each test situation differed considerably for certain chemicals. Unfortunately, soil type and plant species in the field plots were not the same as those in the microcosm; consequently it is not certain how these factors affected the extents of degradation. Elner _et_ _al_. (1981) have studied the movement of a carbamate herbicide, aminocarb, into the surface microlayer of the water column of a tank system and an experimental outdoor pond and found comparable rates of biodegradation following aerial application of the pesticide. Carbonfuran, a systemic insecticide, was found to be degraded slowly in laboratory soil systems (Ahmad _et al_., 1979), leading the authors to conclude that rapid degradation was not the reason for the ineffective control of corn rootworm infestation in one particular soil. Without field calibration of the laboratory system, however, questions arise about the simulation of actual field conditions in the laboratory system, and their conclusion may not be correct.

10. Precautions in The Use of Microcosms

Although many of the virtues of using microcosms in microbiological research have been discussed, potential limitations to microcosm use should be considered, particularly in relation to studying the microbial responses to pollutants. Probably the most obvious problem in microcosm technology is scaling, i.e., how does the isolation of a piece of an ecosystem affect the function of microbial communities, or as smaller and smaller pieces of an ecosystem (scale reduction) are isolated, how many sites within that ecosystem must be sampled before their summarized response or activity equals the whole? There are basically four aspects to this scaling problem: process uncoupling, linear process control, artificial surface enhancement, and specialized compartments within ecosystems. Each poses a problem which may not necessarily be correctable in the laboratory but potential effects on the interpretation of microcosm results should be carefully considered. Certainly microbiologists are far less likely to experience effects due to scaling because microbial populations are so metabolically diverse and, due to rapid growth, are able to compensate for losses of individuals within their populations.

10.1. Process Uncoupling

The fundamental thermodynamic basis of ecosystems involves the generation of energy from the microbial oxidation of degradable organic materials for mobolizing inorganic nutrients produced from primary productivity and geochemical events external to the ecosystem. Ecosystems are viewed as biogeochemical units reflecting the integration of a large variety of metabolic networks. A microcosm, therefore, will house a certain proportion of these networks but not all (Schindler *et al*., 1980). This is diagrammatically shown in Figure 20. A particular microcosm will harbor a certain amount of the biogeochemistry of an ecosystem (size of the microcosm circles), and it will reflect a certain amount of the true dynamics and interactions of an ecosystem (overlap into the ecosystem circle). For most microcosm studies, the circle will be large, indicating an accommodation of many of the metabolic networks found in nature. However, there will be lesser degrees of overlap, indicating limitations or exaggeration of the dynamics of these networks in the microcosm.

Rarely would a situation exist as shown in Figure 20B in which a single microcosm would account, qualitatively and quantitatively, for all the biogeochemistry of the ecosystem. Arbitrary boundary conditions established for an environmental sample, as often occurs in a microcosm, uncouple some of the biogeochemical processes from the ecosystem. Uncoupling may not eliminate any critical components, or change the number and types of microbial interactions, but it could greatly upset the rates at which these interactions occur. A common example of process uncoupling in microcosms occurs when higher trophic levels, such as plankton and grazer communities, are excluded. Goodyear *et al*. (1972) and Jassby *et al*. (1971) have shown, for example, that grazing by herbivorous fish greatly stimulates primary productivity. Flint and Goldman (1975) showed similar responses of diatom communities to grazing by crayfish in fresh-water lakes. Laboratory systems excluding the grazers would give conservative estimates of plankton activity.

A number of studies have shown that bacterivorous animals directly and indirectly affect the growth and metabolic activity (O_2 uptake, nutrient cycling, decomposition rates,) of bacterial populations (Fenchel and Harrison, 1976; Fry, 1982). Hargrave (1970) showed that bacterial respiration in lake sediment cores increased with increasing numbers of the freshwater amphipod, *Hyaletta azteca*. Barsdate *et al*. (1974) demonstrated that certain protozoa, while causing reductions in bacterial density, stimulated their metabolic activity as measured by increased rates of phosphorous uptake. The deposition and processing of animal fecal material is also crucial to the activity of microorganisms in sediment, presumably providing a source of nitrogen (Hargrave, 1976; Lopez et al. 1977). Several investigators have shown that the grazing of detritus-associated bacteria by protozoa and amphipods stimulates the bacterial mineralization of organic detrital material (Barsdate et al., 1977; Harrison and Mann, 1975; Lopez et al., 1977). A typical result using radiolabelled hay as detritus in a seawater system is shown in Figure 21 (Harrison, 1977). Similar types of relationships between grazing organisms and the activity of microbial populations have also been demonstrated in terrestrial systems (Elliott et al., 1979; Stout, 1980). The exclusion of any of these trophic components in a microcosm could therefore significantly alter natural metabolic rates of microbial communities.

In addition, burrowing benthic invertebrates, in the process of reworking sediments and increasing the size of the oxidized zone, cause increased microbial activity (Hylleberg and Henriksen, 1980). Martin and Bianchi (1980), Burney et al. (1981), Straskrabova and Fuksa (1982), and McKinley and Wetzel (1979) have also shown that the metabolic activity of aquatic bacteria is closely coupled to the release of organic materials by growing populations of phytoplankton.

These examples point out that microbially mediated processes in sediment are integrally coupled with a variety of trophic levels in an ecosystem. As discussed already, these couplings may be very sensitive points in the degradative and toxicological response of microbial populations to pollutants.

A good microcosm study should accommodate as many trophic level interactions as possible. In some cases, the size or restrictions of microcosms in the laboratory will preclude the presence of certain trophic levels, particularly fish species and large invertebrates that are not only difficult to maintain in microcosms (due to poor renewal of natural food sources) but are also voracious feeders, often totally denuding the physical structure of the microcosm components. Gillette and Gile (1976), for example, found that a meadow vole in a terrestrial synthetic community study totally consumed all the vegetation and completely turned over all of the soil. Brockway et al. (1979), using pond microcosms, found that snails introduced functional instability to the system and had to be removed. The only way to accommodate some of these higher trophic levels, other than using impratical large tanks, is to add these macrofauna to self-sustaining microcosms for only short experimental periods and then observe the changes in function and structure of the microcosm ecosystem as if it were a zero-order effect. In other cases, intact cores (from 3-30 cm in diameter) from soil or sediment will naturally contain large numbers of macrofaunal organisms. A mixed culture study using sediment slurries in a shake flask cannot accommodate the important biogeochemical couples with different trophic levels.

A second type of process uncoupling involves physical rather than biological factors. Large bodies of water, for example, experience physical mixing and turbulence phenomena which are difficult to model in laboratory tanks and are generally ignored. They may have a significant effect, however, on the process rates of microbial communities. Thermoclines in freshwater lakes are difficult to simulate in microcosms (Harte et al., 1980). It is very complicated in the laboratory, therefore, to investigate the interchange of inorganic nutrients, microorganisms, and organic substrates among the hypolimnion and the epilimnion, as well as the profundal and littoral zones. Isolating the epilimnion in a microcosm (Jassby et al., 1977), without taking into consideration the inputs from the hypoliminion, could produce misleading rate information about microbial processes contained therein. Dissolved oxygen concentration in the hypoliminion, for example, is inversely related to the distance from the sediment (Jones, 1982). Modeling each zone separately in a microcosm and studying output responses from a variety of inputs, including pollutants, may provide enough information to model the interactions and feedbacks of the zones mathematically. Jones (1982) has reviewed some of the sediment coring methods that could be used in these microcosm studies.

Actual turbulence and water velocity of lotic environments are often difficult to accommodate in microcosms, and their absence may affect the natural behavior of microbial communities. Leaf litter is a dominant component of the coarse particulate organic matter in woodland streams. It commonly accumulates at the leading edge of various obstructions, i.e., rocks, wood, debris jams, where it forms into packs due to the current. The processing of these leaf packs to fine particulate organic matter occurs through physical leaching and the combined action of "shredders" in the invertebrate communities and decomposition by colonizing microorganisms (Peterson and Cummings, 1979). Stream current is important in controlling the leaf processing rate since it supplies oxygen, continuously adds new matter, removes decomposed materials, and provides a proper habitat for the invertebrates. The absence of a current in a laboratory microcosm leads to the development of invertebrate and bacterial communities more typical of pool areas in stream where litter decomposition is much slower (Cummins, 1974). In a similar situation, McIntire et al. (1975) have summarized evidence that demonstrates the dependence of primary productivity in periphyton assemblages associated with rock surfaces on the stream current. For example, periphyton productivity is very susceptible to the exhaustion of certain dissolved gases and nutrients; the gradients established for these materials are generally controlled by stream turbulence (McIntire, 1966). Likewise, dislodgment of periphyton from rocks, possibly by the scouring action of suspended solids, greatly promotes respiration and may account for the resulting higher periphyton biomasses in rapidly flowing streams (McIntire, 1978). It is apparent, therefore, that any microcosm design which ignores these mechanical features will likely give a false picture of natural process rates.

Some of the best and most extensively tested microcosms have been developed to study stream ecology. In many cases, adequate simulation of currents and turbulence has been mechanically achieved, providing ecosystem behavior in excellent agreement with field measurements (McIntire, 1964; Gee and Barnik, 1969; Lauff and Cummins, 1964; Warren and Davis, 1971). These studies have provided ideal, but largely untested, laboratory systems for assessing the relationship between the fate and effects of pollutants and the activity of microbial communities. Hansen and Garton (1982) and Maki (1980) have shown their potential use in assessing the toxicological effects of the pesticides diflubenzuron (a chitin synthesis inhibitor) and trifluromethylnitrophenol (a lampricide), respectively. Giesy (1978) has demonstrated, using a microcosm, the toxicity of cadmium to leaf litter colonization of bacteria and fungi and to its eventual decomposition. Bott et al. (1976) have examined the fate of NTA in microcosms. In each example, modeling of stream current and turbulence was critical to the environmental significance of the results.

In the excellent series of studies with marine microcosms and the dynamics of plankton communities, Perez et al. (1977) and Nixon et al. (1979) have shown that light intensity and turbulence were important in determining plankton biomass and realistically modeling the couple between benthic and pelagic phases. Turbulent mixing of the water in their 150-liter tanks was obtained with specially designed paddles made of a plastic grid (12 mm x 12 mm openings) and driven by electric motors which reversed direction every 30 seconds. Water was also passed across the sediment surface (contained in a plastic box) at a rate of 1.4 cm sec^{-1} (water in the tank was exposed to the sediment once every six days), using an air displacement pump and thereby eliminating any functional gradients. Despite considerable precautions, the microcosm generally underestimated turbulent mixing in the field. It is not a trivial matter, therefore, in a pelagic model of this type to simulate natural mixing and turbulance in a microcosm. Depending on the research question being addressed, ignorance of this design feature may result in process rates which are physically uncoupled from normal ecosystem behavior. It may also significantly affect the distribution of toxicant and correspondingly the ultimate fate and effects of a pollutant.

Natural lighting is also a complicated physical factor to model in the laboratory systems. As indicated before, the phytoplankton, epiphyte, and rooted macrophyte communities in many types of ecosystems can influence the metabolic rates of microbial communities. This is due to their excretion of organic material, their contribution to decomposable organic detritus, and their role as a food source for zooplankton and benthic macrofauna which elaborate and process degradable fecal material. These plant communities should therefore be included in microcosms. Standard, cool white 40 W fluorescent lights are commonly used to maintain the plant communities. However, Nixon et al. (1979) have shown that light intensities in the microcosm which approximates that seen in the field (Narragansett Bay, Rhode Island) cause "super blooms" of phytoplankton (Figure 22). Intensities of 5 Langleys (ly) per day in the microcosm produced algal populations similar to those observed in the field: light intensities of around 40 ly/day were observed, and greater than 40 ly/day initiated an algal bloom. Interestingly, bloom conditions in the microcosms were considerably more dramatic than in the field; "instantaneous" growth rates of 0.23 day^{-1} (25 ly/day) and 0.03 day^{-1} (>40 ly/day) were obtained, respectively. Explanations for these differences may lie in the spectral quality of natural light and its absorption characteristics in natural waters. Scaling of light intensity and quality (spectral distribution) therefore, is essential if the natural activities of plant communities and their dynamic interactions with microbial communities are to be studied and perhaps related to pollutant perturbations.

A final microcosm design feature which can cause process uncoupling is the use of a closed microcosm when the environmental situation being modeled is quite open. In closed systems, microcosms are ecosystems unto themselves, having self-sustaining properties and nutrient cycling dynamics which are unique environmentally but conceptually within the realm of the general ecosystem theory (Hill and Weigert, 1980). This concept may be true except that in a closed system, the equilbrim state will be a function of the initial conditions. The chance of this equilibrim state being an equivalent with some environmental counterpart is remote. Process rates therefore, may be greatly exaggerated (faster or slower) and essentially uncoupled from the natural ecosystem function. In an open system, however, the equilbrium state is a function of the inputs and outputs, and, regardless of which portion of an ecosystem is modeled in a microcosm, it must experience a set of inputs and outputs similar to what it would receive in the field. This consideration of inputs and outputs in microcosm study is unfortunately ignored in many cases.

inputs, typical field conditions, can often be modeled by continuous-flow systems in which the water source is taken directly from the environment under study. Thus, a continual input of microbial, algal, and invertebrate larval biomass can be achieved in the laboratory. Organic and inorganic nutrient inputs and outputs can also be modeled, thereby preventing growth limitation due to nutrient depletion or to the buildup of toxic endproducts that would normally be associated with a closed system. Lassiter (1979) has proposed that continuous culture theory be applied to open microcosm studies. DePinto et al. (1980) have demonstrated the value of a continuous-flow system relative to a closed system in studying techniques for acid lake recovery.

10.2. Linear Process Control

As stated earlier, one of the criteria for applying process rate data from mixed culture studies of microbial communities in the laboratory to actual field situations is the verification exercise showing the applicability of the microcosm data to the field. It is then logical to ask how generally applicable this verification is. Can similar results be obtained with other compounds and in other types and designs of microcosms when considering other parts of the same ecosystem, or similar ecosystems of different geographic location, or completely different ecosystems? The development of kinetic expressions for biodegradation provides a relevant example. Assume that a chemical degrades much more rapidly in a shake flask test containing a sediment water slurry than it does in one containing only water. Since it is often difficult to characterize quantitatively the degrading microbial populations associated with sediment, the development of kinetic expressions involving rates per cell is likewise difficult. As a surrogate, the degradation rate can be related to the amount of organic detritus available, assuming that the distribution of bacteria on each particle is approximately the same. This is possible for the degradation of a number of pesticides in sediment and soil where a biologically mediated binding to particulates is the principle degradation process (Katan et al., 1976; Pritchard et al., 1979). A kinetic expression relating binding rate to the mass of detritus, which has been successfully used to describe the biological fate of a pesticide in a sediment-water microcosm (Pritchard et al., 1982), means that with a sediment sample from any similar environment the biodegradation rate can be predicted by knowing the average detrital concentration and the binding rate constant. This assumes the relationship of detritus concentration to degradation rate is linear over a large range of detritus concentrations. Studies in our laboratory (Cleveland, 1982) have shown that the relationship for estuarine sediments is not entirely linear (Figure 23). Binding of methyl parathion to three different sediment types reached saturation at high detritus concentrations. Verification of the binding rate expression in microcosms was at the lower detritus concentrations (Pritchard et al., 1982). The question of which type of environmental situation, or, since the environment cannot be dosed with the chemical, which type or size of microcosm is required before the effect of this nonlinear relationship can be perceived and verified must be answered. The answer is not obvious and may come only from a large set of empirical experiments, such as testing a whole gradation in size and complexity of microcosms.

In most research settings, scaling experiments are not possible. It is enough to be able to adequately describe the events in a single microcosm. However, some scaling work will eventually be necessary and it is hoped that results from a few experiments will have general application. In the meantime, careful attention must be paid to checking the linearity of kinetic expressions to at least become aware of where problems in extrapolation might be expected.

10.3. Wall Effects.

The growth of bacteria and other epiphytes on the walls of the microcosm container can potentially have dramatic effects on the experimental results. In many microcosm studies, this problem is unfortunately overlooked or in many cases simply ignored. Generally, within hours to days, a microbial film begins to develop on the walls of any container used to house natural water or sediment samples. A succession of bacterial types usually results, and colonization by protoza, diatoms, and other algae generally follows. Long-term incubations, typical of many microcosm studies, can produce enough wall growth to represent a substantial part of the biology in the microcosm. Dudzik _et al_. (1979), for example, have calculated that a close-packed 1 μm layer of algal cells on the walls of a microcosm tank 1 m in diameter and 1 m deep has about the same volume density of phytoplankton in a small mesotrophic lake. Release of metabolic end products, including polymeric materials, death and decay of cells, and sorption of organic and inorganic materials are all consequences of wall growth which can greatly influence carbon and nutrient cycling rates in a microcosm.

Several aproaches can be used to accommodate the wall growth phenomena. It definitely cannot be ignored. Continual scraping of the vessel walls is a feasible approach (Perez _et al_., 1977), but this procedure often leads to unnaturally high concentrations of suspended solids or the deposition of large amounts of degradable organic material on the sediment surface. Depending on the research question being asked, the effects of this additional organic loading must be reconciled in the interpretation of microcosm results.

Harte et al. (1979) have surmounted the wall growth problem by periodically transferring the contents of a microcosm to a clean vessel. Although this is potentially feasible in a microcosm designed to model the epilimnion of a lake, it is not very practical for systems with sediment or with other highly structured components.

A more reasonable approach, in the authors' opinion, is to accept the wall growth as inevitable and then determine its role in the biology and nutrient cycling in the microcosm. This could be accomplished by first adjusting the operating conditions of the microcosm in an attempt to possibly slow the rate of wall growth accumulation. Increasing the flow rate of water through the system (if this is possible) or increasing turbulent mixing might attain this result. Second, by hanging glass or plexiglass slides in the microcosm, it should be possible to surrogately sample the wall growth and examine or measure the associated microbial activity. These results can then be quantitatively factored into the interpretation of the microcosm results.

10.4 Specialized Compartments within Ecosystems.

The object of a microcosm study, as already discussed, is to capture certain portions of the ecosystem which the investigator feels could be critically important in the overall assessment of the response of a microbial community to a pollutant. What constitutes or defines a "portion" of the ecosystem? This is a complicated question that will affect assumptions in designing a microcosm and will cause problems in extrapolation. Take for example the cross section of an estuarine ecosystem shown in Figure 24. An assumption in modeling the ecosystem in a microcosm may be to establish a sediment/water system representing the average sediment surface area to water volume ratio of the bay. This assumption may be tenable for some studies but from the standpoint of exposure assessment in which a chemical may biodegrade much more rapidly in sediment than water, it is not tenable since the chemical will disappear at a faster rate in shallower waters where it encounters sediment more frequently. Thus the microcosm results may give a conservative estimate of fate because of this original assumption.

To elaborate further, biodegradation may also be more rapid in and around rooted macrophytes, such as found in grass flat areas and adjoining salt marshes. Higher concentrations and activities of bacteria on the plant leaf surfaces, in the root rhizosphere, and in the associated detritial material may contribute significantly to the biodegradation of certain chemicals. This component of pollutant biodegradation has been virtually ignored over the years, yet its potential contribution would seem to merit further experimental consideration, particulary in microcosm studies. Hsu and Bartha (1979) have shown in soil systems that bacterial populations associated with the roots of plants degrade parathion faster than populations from areas without these plants. A microcosm designed, therefore, to model only the more geographically dominant neritic component of the estuarine system would ignore this contribution from the plants. Thus, if this type of microcosm is used to study the fate of a chemical in an estuary, estimation of the chemical's fate in the whole ecosystem would be erroneous. Other areas of estuaries would also appear to be unique: transition zones between fresh and salt water where sediment resuspension could greatly affect biodegradation rates and certain embayments where different organic and nutrient loadings, including anthropogenic sources, could affect fate processes significantly. Similar considerations can be cited for lakes, rivers, and terrestrial environments.

Where does one draw the line? How many microcosm studies will be needed to adequately describe the response of microbial communities to a pollutant, especially in aquatic systems where exposure is maximized by water?

mixing? There are three potential solutions to this problem. First, a hydrodynamic mathematical model of the particular environment of concern can be used to estimate the residence time of a chemical in a perceptually unique part of an environment. The EXAMS model (Burns et al., 1982) is an excellent generalized model which can be applied to a large variety of aquatic systems. If the residence time in one of the subsidiary parts of an ecosystem (a grass bed area for example) is short relative to a major part of the ecosystem (the water column), then even though the former part may have a considerably greater biodegradation potential than the latter, its effect will go unnoticed because of hydrodynamic conditions of the environment. Consequently, the number of unique parts of an ecosystem that need to be considered for experimentation can be reduced.

A second solution would require actual experiments with each perceived unique part of an ecosystem in microcosm studies, establishing a ranking of parts that had the most significanct responses of the microbial populations and then assuming that their relationship to one another remained constant from one ecosystem to the next. For example, bacterial populations associated with plant roots might only stimulate the degradation of a toxicant by a factor of two relative to the activities in sediment. This is not significant enough in an estuarine ecosystem to merit further consideration since the relative mass of sediment and its firsthand exposure to the pollutant are considerably higher. On the other hand, a factor of 5 to 10 would probably be significant. Once this relationship is established, there will probably be few exceptions. For each significantly active part, its overall contribution to the ecosystem can again be assessed through hydrodynamic modeling.

Finally, a third solution, which is perhaps the most difficult but the most useful, is to characterize the factors which make one unique part of an ecosystem more microbially active than another. This type of characterization would come from a combination of microcosm and mixed culture tests as already discussed. For example, if an observed increase in biodegradability of a pollutant associated with the microflora of rooted macrophytes could be attributed to larger degrader populations, then if the biodegradation rate constant (degradation rate per cell) of a pollutant and the concentration of degraders in each particular unique part of an ecosystem are known, the biotic fate of a pollutant can be assessed. This doesn't really reduce the number of initial microcosm studies, but it allows the consistency of the relationship to be checked from ecosystem to ecosystem. More importantly, it eliminates the need to conduct microcosm studies in several sites within a unique part of the ecosystem as a check on the variability of the recorded responses.

Table 1. Physical Parameters, Kinetic Expressions and Rate Constants for Fate Processes Used To Describe the Distribution and Degradation of Methyl Parathion in a Continuous-flow Microcosm.

	Water	Sediment[a]			
A. Physical Parameters	Column	1	2	3	4
Depth (cm)	7.5	0.2	1.0	1.0	2.5
Volume (ml)	600	78.5[b]	78.5[b]	77.3[b]	16.9[b]
Diffusion cofficient (cm^2 sec^{-1})	c.m.[c]	0.1	0.1	0.05	0.05
Detritus (gm)	0	32.8	76.4	57.2	0
Sand (gm)	0	0	0	240	721
Porosity (gm/ml)	1.0	0.97	0.92	0.60	0.25
Hycroxyl concentration ($\times 10^7$)	3.5	0.63	0.63	0.62	0.63
Partition cofficient					

B. Process	Kinetic Expression[d]	Rate Constant
Alkaline Hydrolysis	$= -K_{hyd} [OH^-]c$	-2×10^4 M hr^{-1}
Biodegradation (water)[c]	$= -K_{Bio-w}\ c$	0.007 hr^{-1}
Biodegradation (sediment)	$= -K_{Bio-s}\ C[S]$	0.004 ℓ/gm·hr
Sorption rate[f]	$= -K_{ADS}\ D[S]$	instaneous
Equilibrium Partitioning	$K_{oc} = \quad [OM]^{-1}$	600 ℓ/kg

[1] Sediment segment 1 is top most segment

[b] Interstitial water

[c] c.m. = completely mixed

[d] c = chemical concentration; [S] = sediment concentration (mg/ℓ); KOC = equilibrium partition cofficient normalized against organic matter associated with sediment; C sorb = equilibrium concentration of sorbate on seediment; C diss = equilbrium dissolved sorbate concentration; [OM] = fractional mass of organic carbon in sediment.

[e] Biodegradation in water was treated as a first order reaction since concentration of bacteria in microcosm and flask studies was equal.

[f] Adsorption and desorption rates are assumed to be completely reversible.

Table 2. A Summary of Kinetic Expressions Used To Describe the Cycling of Ammonia in Natural Systems and To Exemplify the Tight Coupling of Associated Processes with Oxygen Availability.

$= S \; k_1 I \, [O_2] \, [P_3] \, [ORG\text{-}N] \, [NO_3^-]$	(1)	Anaerobic decomposition of organic nitrogen using nitrate reduction (denitrification)
$+ \; k_2 \, I \, [O_2] \, [P_4] \, [ORG\text{-}N] \, [NO_2^-]$	(2)	Ibid, using nitrate reduction
$+ \; k_3 \, [P_3] + k_4 \, [P_4] \;\; [ORG\text{-}N] \, [O_2]$	(3)	Aerobic decomposition of organic nitrogen
$- \; k_5 \, [P_n] \, [NH_4]$	(4)	Nitrogen fixation
$- \; k_6 k_7 \, [P_1] \, [NH_4] \, [O_2]$	(5)	Production of organic-nitrogen through assimilation of ammonia
$+ \; k_8 k_9 \, [P_2] \, [NO_2^-] \, [O_2]$	(6)	Ibid, for nitrate
$+ \; k_{10} k_1 \, I \, [O_2][P_3] \, [ORG\text{-}N] \, [NO_3]$	(7)	Assimilation of nitrate during anaerobic decomposition of organic-N
$+ \; k_{11} k_{12} \, I \, [O_2] \, [P_4] \, [ORG\text{-}N] \, [NO_2]$	(8)	Ibid, for nitrate
$- \; k_7 \, [P_1] \, [NH_4] \, [O_2]$	(9)	Direct nitrification of ammonia to nitrite

Assumptions:

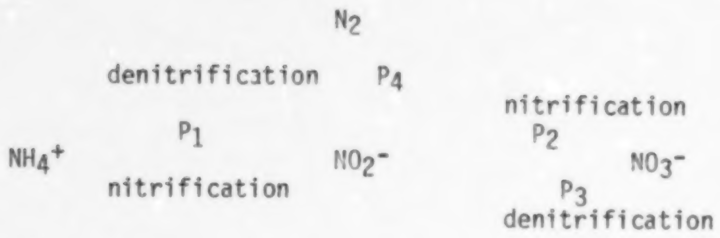
a. The synthesis of organic nitrogen material (ORG-N) use nitrogen obtained from four sources; ammonia, nitrate and N_2 fixation (equations 4-8). Organic nitrogen was a fixed portion of total organic matter; 10 moles of N atoms per mole of organic material. Appearance and disappearance of organic nitrogen was dynamically expressed as

$$\frac{d[ORG\text{-}N]}{dt} = \Sigma \, k_d[P] - \Sigma \, k_b[P]I[O_2][ORG\text{-}N]$$

where K_d = death rate of any microbial population [P] and k_b = the decomposition (aerobic and anaerobic) rate of microbial population [P] as a function of oxygen availability ($I[O_2]$).

b. Ammonia is used as a suppressant for N_2 fixation (equ. 4).

c. Microbial population sizes (P_1, P_2, P_3, P_4, P_n) are fixed and of equal chemical composition. P_n is the nitrogen fixing population that is active only when nitrate and ammonia are scarce. The populations are associated with the following nitrogen transformation steps.



d. Oxygen is a forcing (time-varying) function causing reactions to be turned on or off. Oxygen inhibition functions (I), which could mean, for example, actual inhibition, such as inhibition of denitrification by the presence of oxygen, or it could mean a preference, such as inhibition of NO_3 uptake by microorganism in the presence of "preferred" NH_3, were expressed kinetically as:

$$k = k_{max} e^{-ax^2}$$

where k is the rate cofficient of the inhibited process, x is the concentration of inhibiting substance and a is a scaling cofficient.

e. S = a stoichiometric cofficient, k's are rate coffcients for various processes (subscripts have no value for identifying the processes to which they belong).

f. Assimilation of nitrate (equ. 7), ammonia (equ. 5) and nitrite (equ. 6,8) accompanies microbial processes in which biomass generation is energetically feasible (aerobic decomposition, denitrification and nitrification). Assimilation terms for both ammonia and nitrate are represented as proportional to the product of the energy yielding process and the concentration of NH_3.

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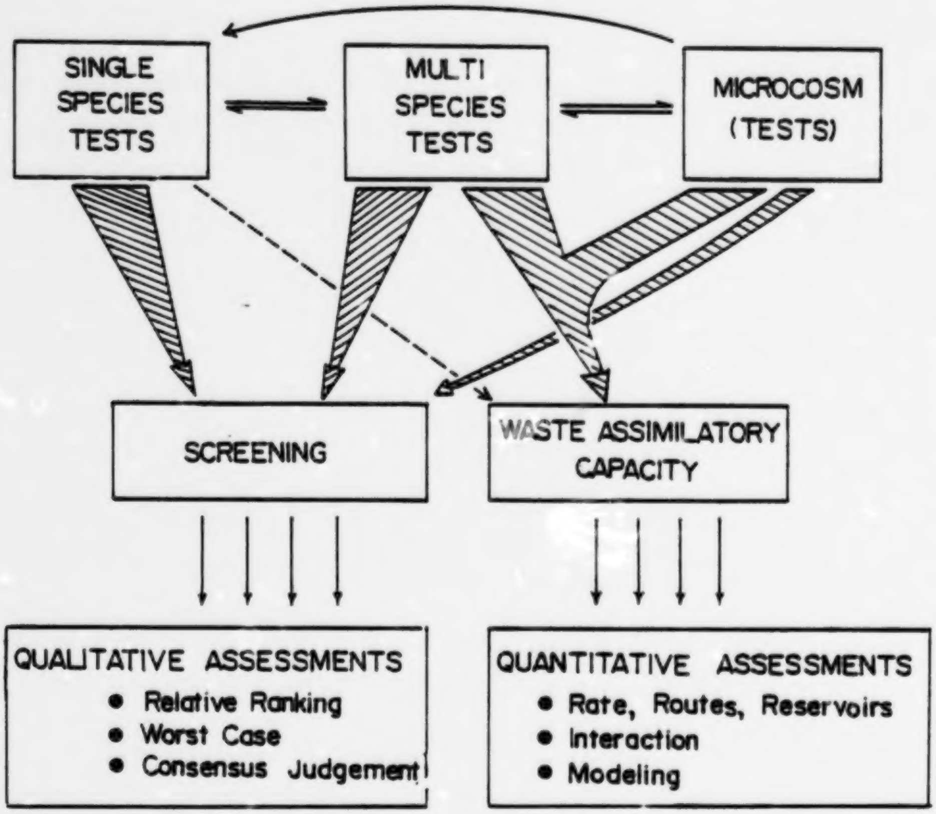
SINGLE
SPECIES
TESTS
MULTI
SPECIES
TESTS
MICROCOSM
(TESTS)
SCREENING
WASTE ASSIMILATORY
CAPACITY
QUALITATIVE ASSESSMENTS
• Relative Ranking
• Worst Case
• Consensus Judgement
QUANTITATIVE ASSESSMENTS
• Rate, Routes, Reservoirs
• Interaction
• Modeling

Figure 1. Schematic diagram of possible information flow from laboratory test systems to risk assessments. Differences in tests are explained in the text. Cross hatched areas represent current and projected flow of information.

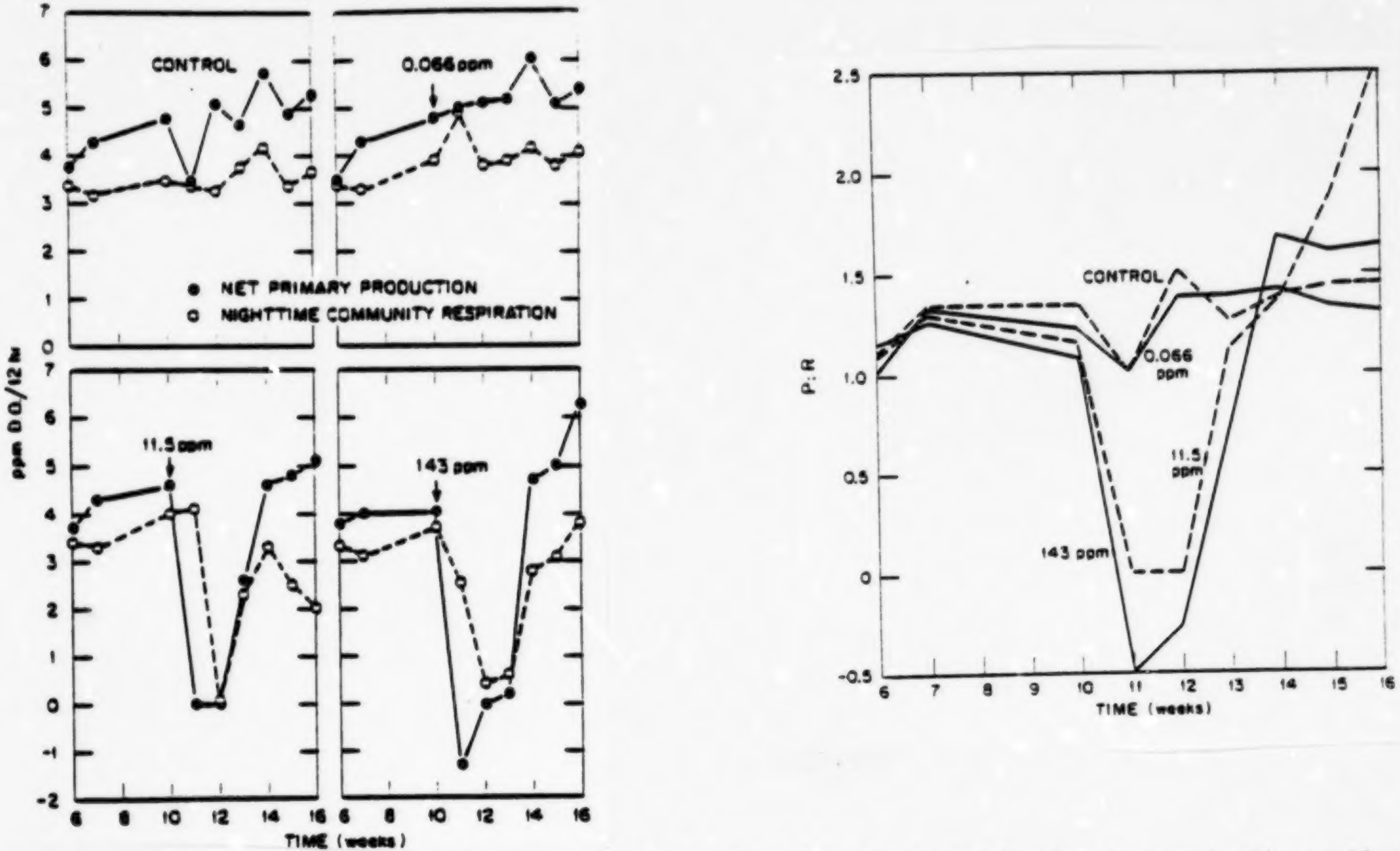

Figure 2. Net production and nighttime community respiration (left) and production to respiration ratios ratios (right) in microcosms at three arsenic levels. Open circles: nighttime community respiration; filled circles: net production. Each point represents the mean of three microcosms. Arsenic was added, in the concentrations indicated, immediately following P and R measurement. (After Gidding and Eddleman, 1978).

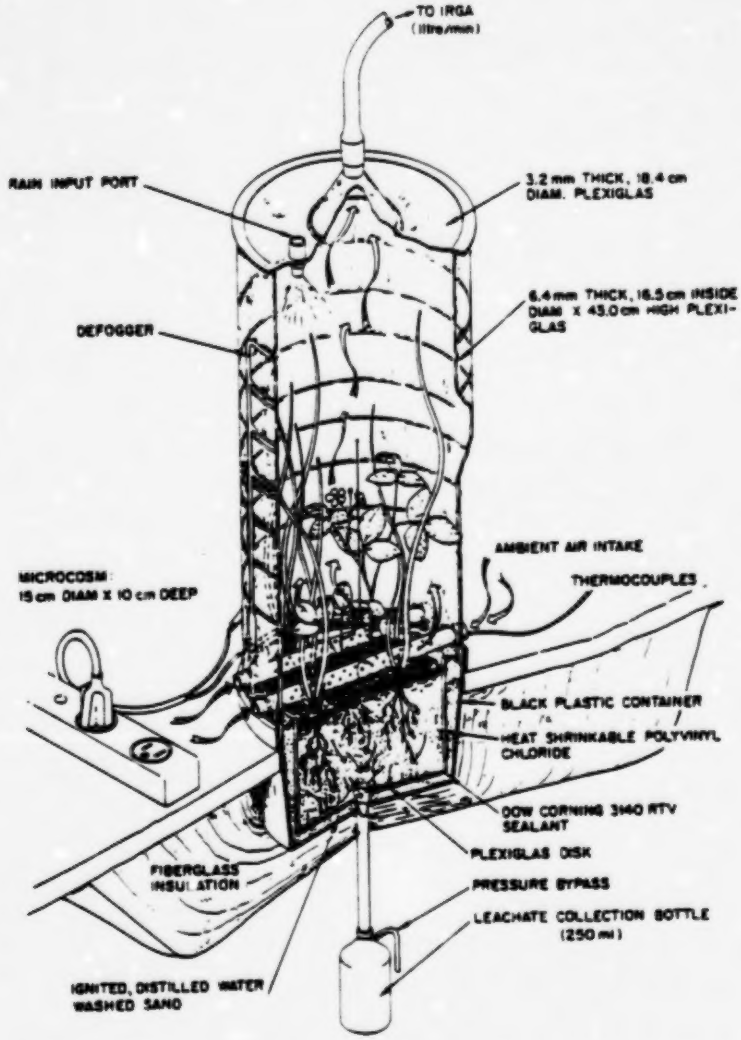
TO IRGA
(litre/min)
RAIN INPUT PORT
3.2 mm THICK, 18.4 cm
DIAM. PLEXIGLAS
6.4 mm THICK, 16.5 cm INSIDE
DIAM X 43.0 cm HIGH PLEXI-
GLAS
DEFOGGER
AMBIENT AIR INTAKE
MICROCOSM:
15 cm DIAM X 10 cm DEEP
THERMOCOUPLES
BLACK PLASTIC CONTAINER
HEAT SHRINKABLE POLYVINYL
CHLORIDE
DOW CORNING 3140 RTV
SEALANT
PLEXIGLAS DISK
FIBERGLASS
INSULATION
PRESSURE BYPASS
LEACHATE COLLECTION BOTTLE
(250 ml)
IGNITED, DISTILLED WATER
WASHED SAND

Figure 3. Cross section of encased and cuvette-covered soil core microcosm showing the two ecosystem-level monitoring points of CO_2 and nutrient export. (After Van Voris et al., 1980).

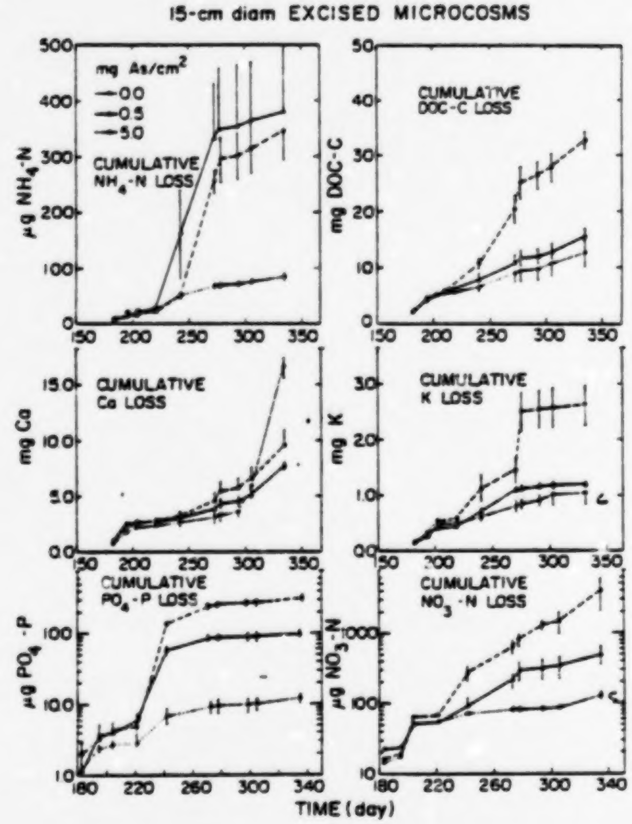

Figure 4. Cumulative nutrient loss from soil core microcosms treated with As (± 1 SE, n = 3). (After Jackson et al., 1979).

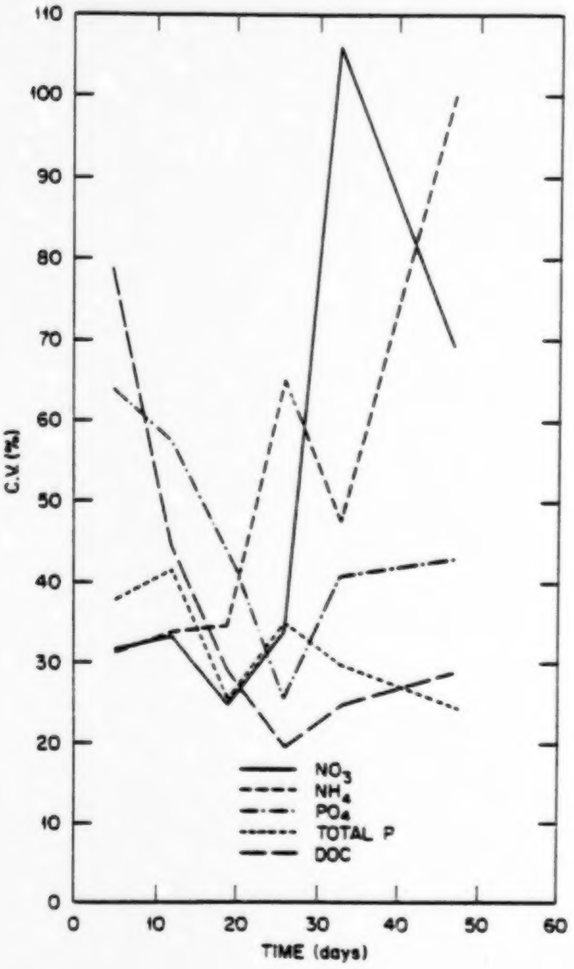

Figure 5. Cofficients of veriation (percentages of tweleve replicate microcosms) of nitrate, ammonium, phosphate, total phosphorous and dissolved organic carbon concentration intweleve replicate 70 liter pond microcosms. Microcosms contained pond water, sediment and a macrophyte community (After Harris, 1980).

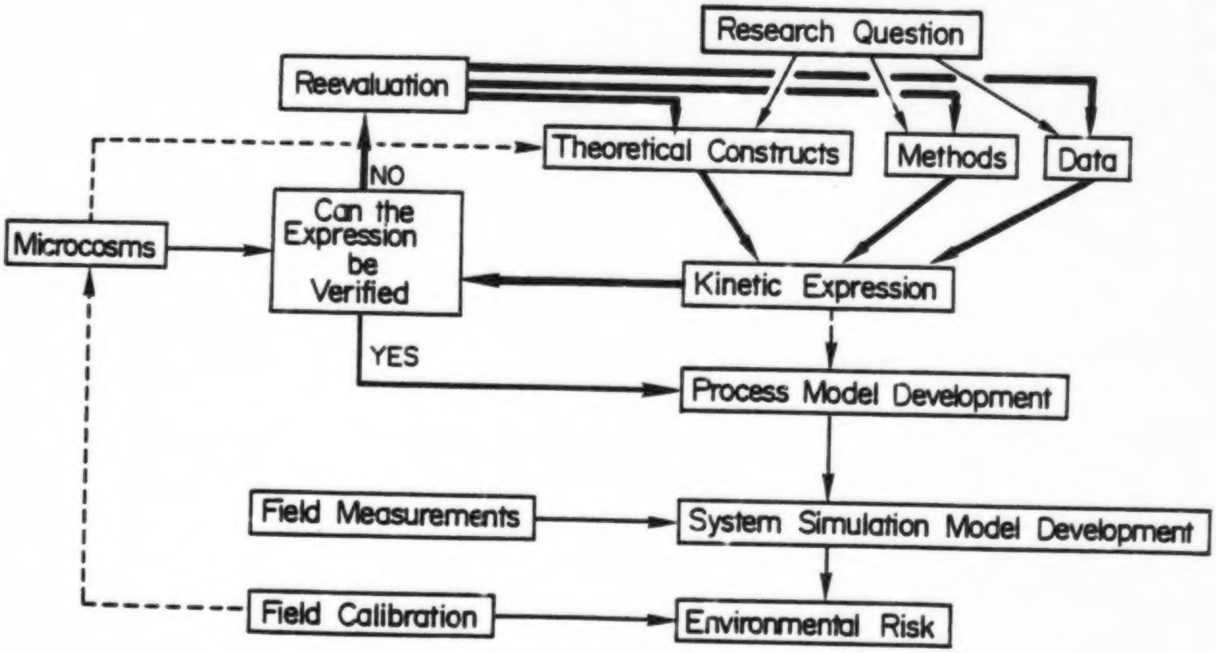
Research Question
Reevaluation
Theoretical Constructs
Methods
Data
NO
Can the Expression be Verified
Microcosms
Kinetic Expression
YES
Process Model Development
Field Measurements
System Simulation Model Development
Field Calibration
Environmental Risk

Figure 6. Flow diagram depicting the use of microcosms as verification tools. The basic iteration involves verifying the conceptual and experimental basis of the kinetic expressions using complex natural situations such as microcosms. Once the kinetic expressions are verified they can be used in ecosystem simulation models.

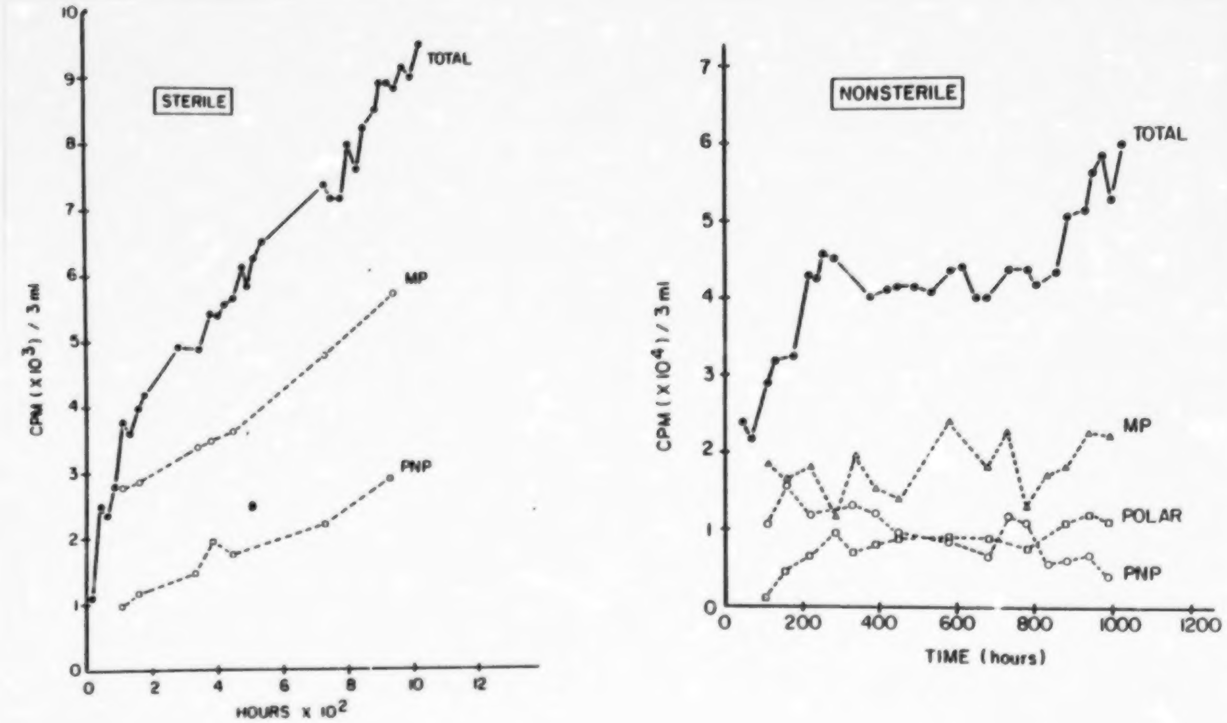

Figure 7. Concentration of total radioactivity (solid line), methyl parathion (MP), paranitrophenol (PNP) and unextractable radioactivity (polor) in water overlying sediment in sterile and nonsterile microcosm systems. Unextractable radioactivity refers to radioactive products remaining in a water sample after two extractions with ethylacetate under acidic conditions. Flow rate of sea water into microcosm was 0.35 l/day giving a retention time of 48 hours. Inflow concentration of radioactivity was approximately 12,000 cpm/3ml or 20 µg/l methyl parathion. Sterile microcosms were initially sterilized by adding formalin to a concentration of 2% v/v. The presence of 0.1% formalin in the inflowing toxicant solution (sterile reservoir) was sufficient to maintain sterility in the reaction vessel even with continuous addition of unsterilized seawater. (After Pritchard et al., 1982.)

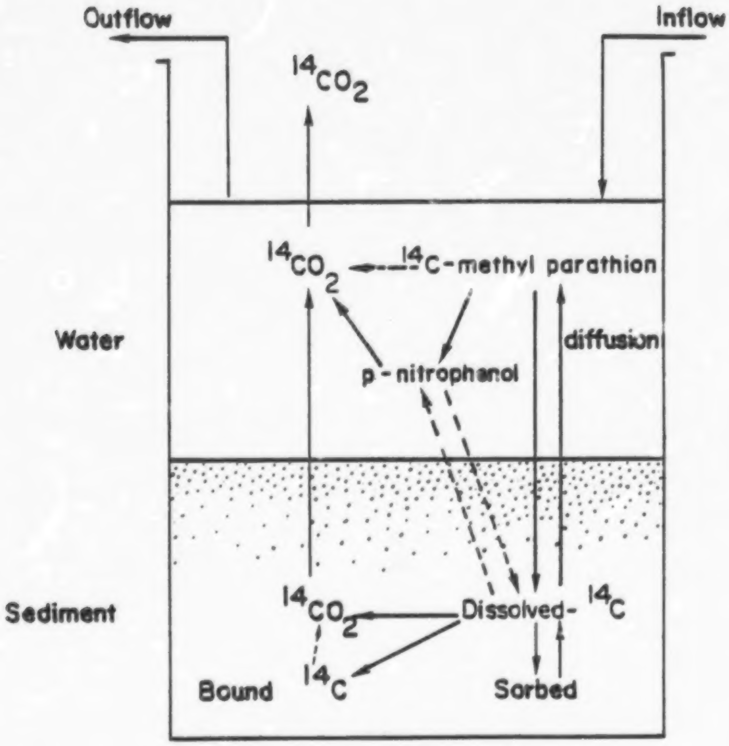
Outflow
Inflow
$^{14}CO_2$
$^{14}CO_2$
^{14}C-methyl parathion
Water
diffusion
p - nitrophenol
Sediment
$^{14}CO_2$
Dissolved- ^{14}C
Bound
^{14}C
Sorbed

Figure 8. Conceptual description of mathematical model developed to describe the distribution of radioactivity, methyl parathion, and its degradation product paranitrophenol in the microcosm. It requires kinetic inputs on hydrodynamics, diffusion in to sediment, adsorption, desorption, partition coefficients, irreversible binding to sediment, abiotic degradation, biotic degradation and biological mineralization. A mathematical equation for each of these proceses has been formulated (See Table 1).

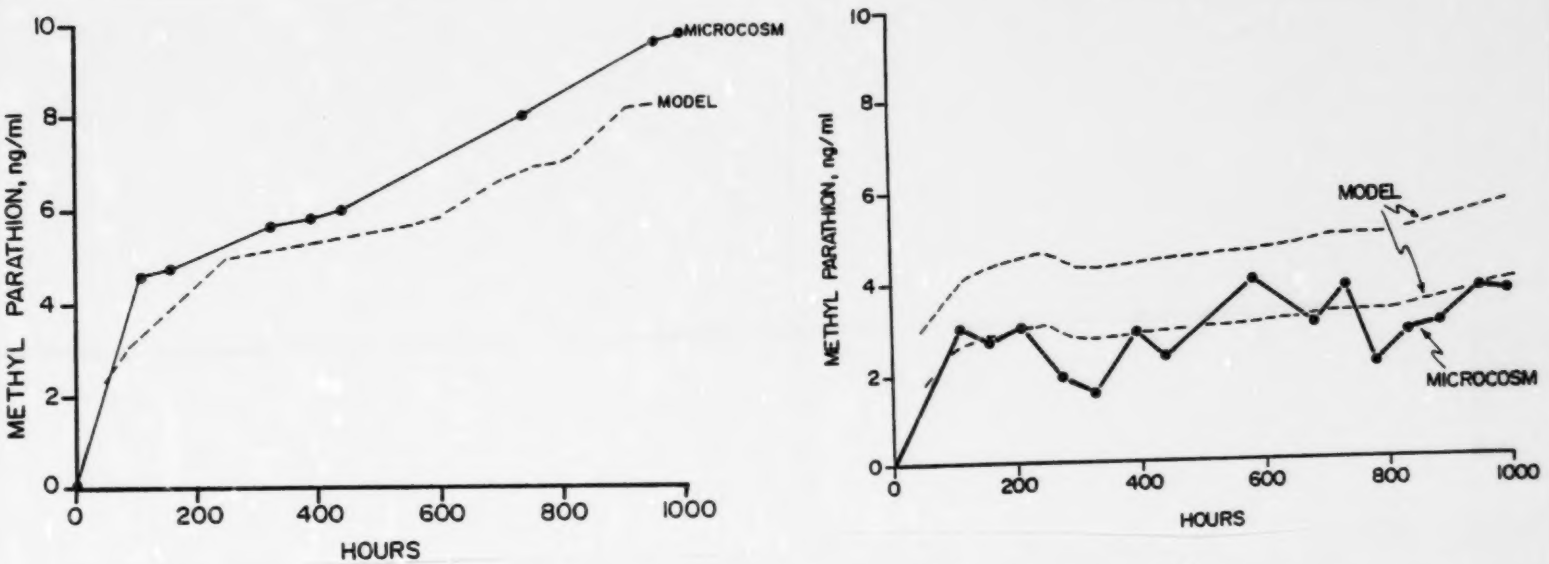

Figure 9. Comparison of changes in methyl parathion concentration in the water column (solid line) of the sterile (left) and nonsterile (right) microcosm (solid line) with the predicted response of the microcosm model (dotted line). Model prediction was based on rate coefficients derived from shake flask studies (containing sediment-water slurries) on sorption rates partitioning to sediments and biotic and abiotic degradation rates. An abiotic degradation rate constant of 2.0×10^4 M [OH] hr^{-1} and biotic degradation rates in water and sediment of 0.0006 hr^{-1} and 0.002 l/gm·hr respectively were used in the model. A hypothetical biodegradation rate of 0.008 l/gm·hr was used in the second model line in the nonsterile microcosms to fit the data more closely. Methyl parathion concetrations were taken from Figure 7 (After Pritchard et al., 1982).

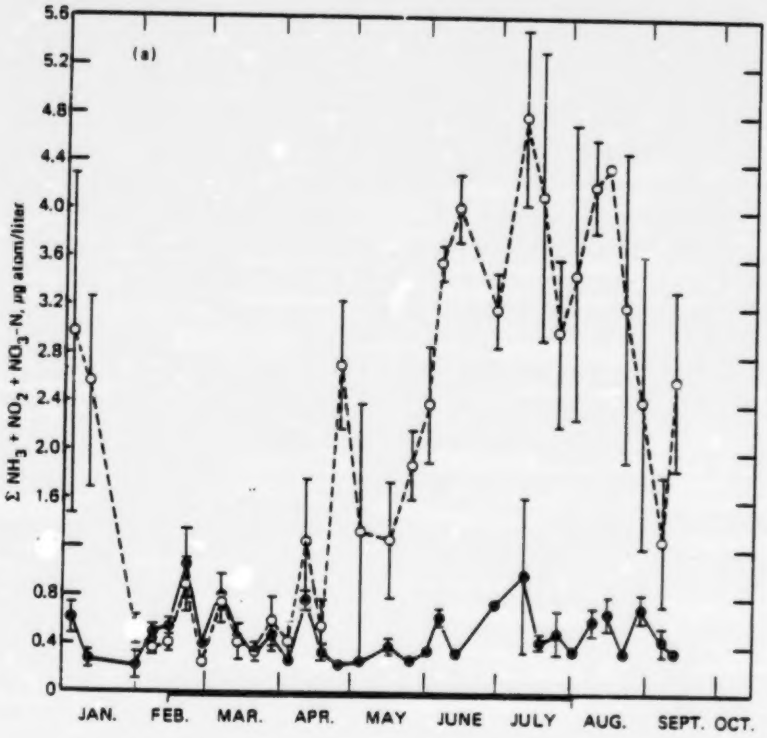

Figure 10. Average concentration of total inorganic nitrogen per liter (± standard error) in the three oiled tanks (●—●) and the three control tanks (○---○). Heavy line on the time axis denotes the period of oil addition. (After Elmgren et al., 1980).

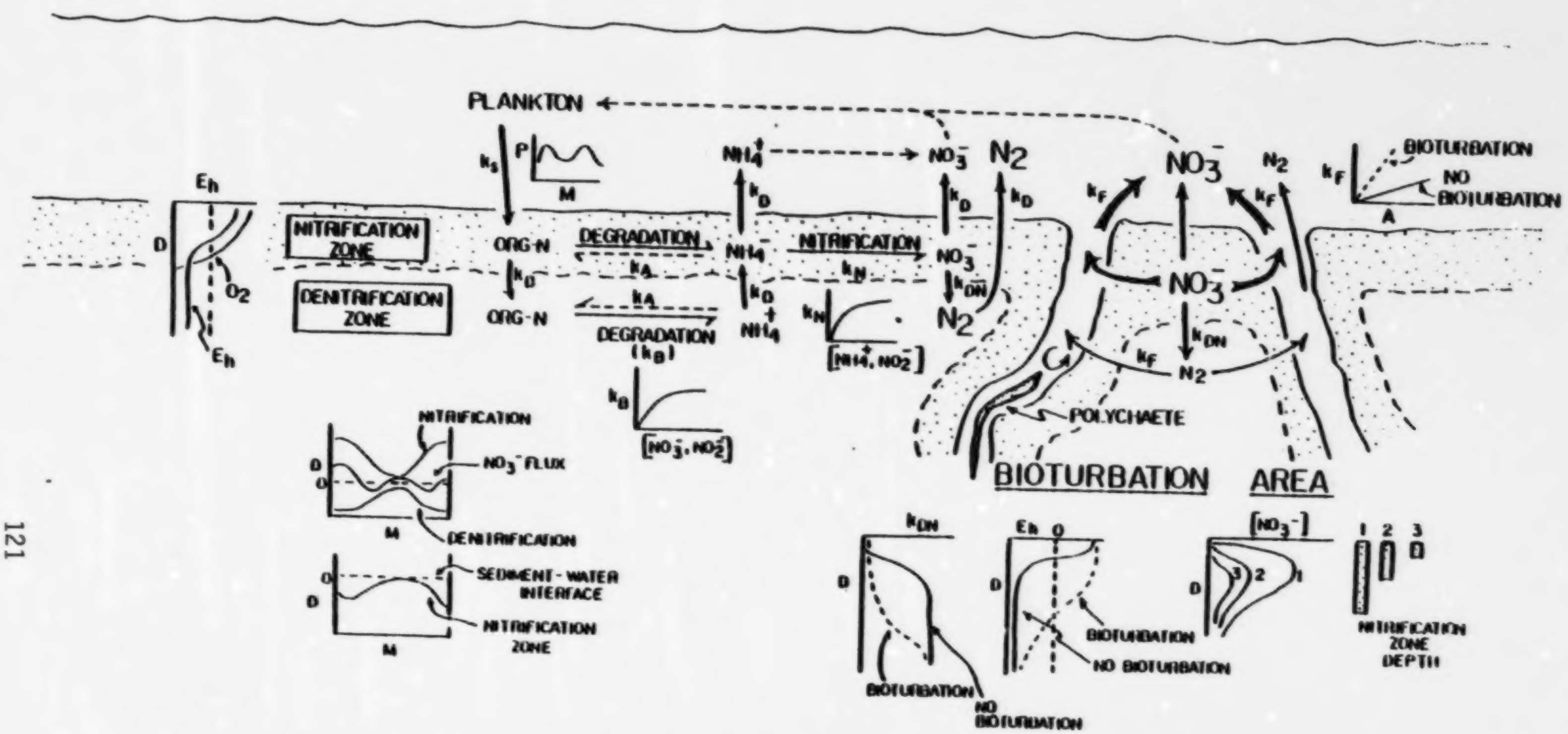
PLANKTON
k_s
P
M
NH_4^+
NO_3^-
N_2
k_D
E_h
D
O_2
E_h
NITRIFICATION ZONE
DENITRIFICATION ZONE
ORG-N
k_D
ORG-N
DEGRADATION
k_A
k_A
DEGRADATION (k_B)
k_B
$[NO_3^-, NO_2^-]$
NH_4^+
k_D
NH_4^+
NITRIFICATION
k_N
k_N
$[NH_4^+, NO_2^-]$
NO_3^-
k_{DN}
N_2
k_F
NO_3^-
k_F
N_2
NO_3^-
k_{DN}
k_F
N_2
POLYCHAETE
BIOTURBATION AREA
k_F
BIOTURBATION
NO BIOTURBATION
A
NITRIFICATION
NO_3^- FLUX
DENITRIFICATION
M
SEDIMENT-WATER INTERFACE
NITRIFICATION ZONE
k_{DN}
BIOTURBATION
NO BIOTURBATION
E_h 0
BIOTURBATION
NO BIOTURBATION
$[NO_3^-]$
3 2 1
1 2 3
NITRIFICATION ZONE DEPTH

Figure 11. Conceptual diagram of the nitrogen cycle emphasizing its relationship to the physical integrity of the natural systems and to the activities of higher trophic organisms.

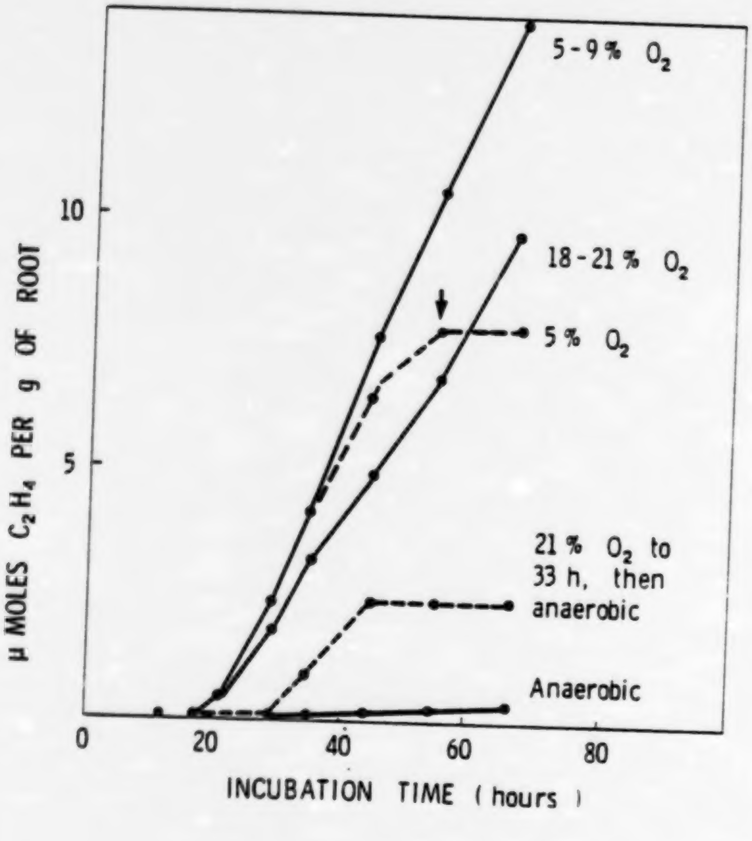

Figure 12. Nitrogen fixation (acetylene reduction method) by washed, excised roots of Spartina alterniflora of various oxygen concentrations. (After Mann, 1981.).

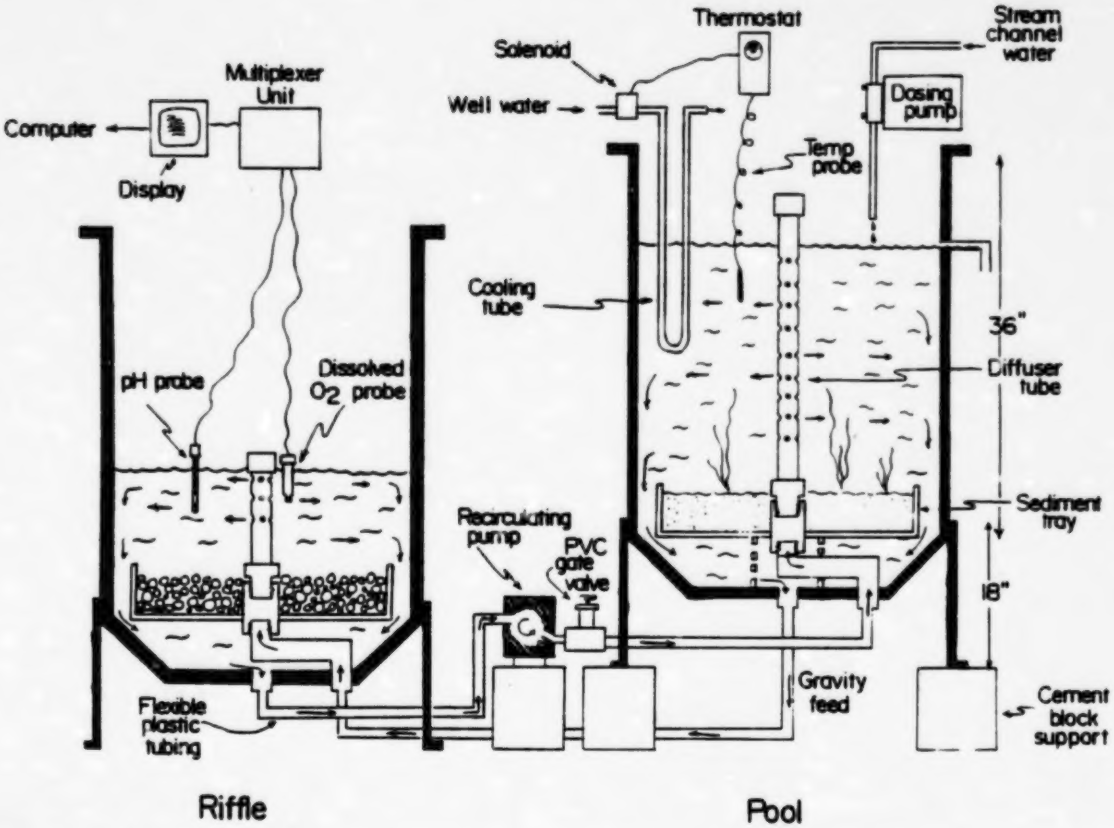
Thermostat
Stream channel water
Solenoid
Multiplexer Unit
Well water
Dosing pump
Computer
Display
Temp probe
Cooling tube
36"
pH probe
Dissolved O_2 probe
Diffuser tube
Recirculating pump
Sediment tray
PVC gate valve
18"
Gravity feed
Flexible plastic tubing
Cement block support
Riffle
Pool

Figure 13. Schematic diagram of microcosms used to model the riffle and pool sections of an outdoor stream channels. Each tank holds 80 stream water. Microcosm are operated as closed systems in which recirculation and turbulance generation are accomplished through the use of a diffuser tube and a water pump. Trays are filled in the field and allowed to acclimate there for two weeks prior to their transfer to the microcosms (after Pritchard, 1982).

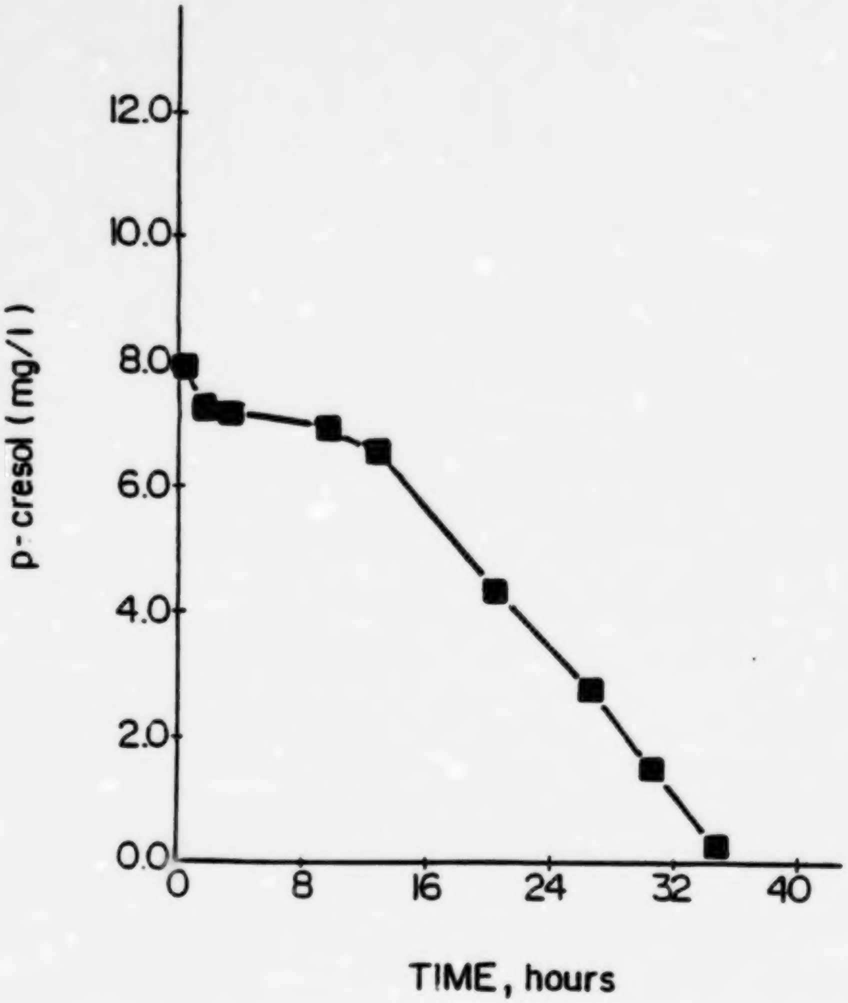

Figure 14. Rate of disappearance of p-cresol (initial concentration, 8 mg/ℓ) in a riffle-pool microcosm. Microcosm was operated as closed continuously mixed ractor. (After Pritchard et al., 1982).

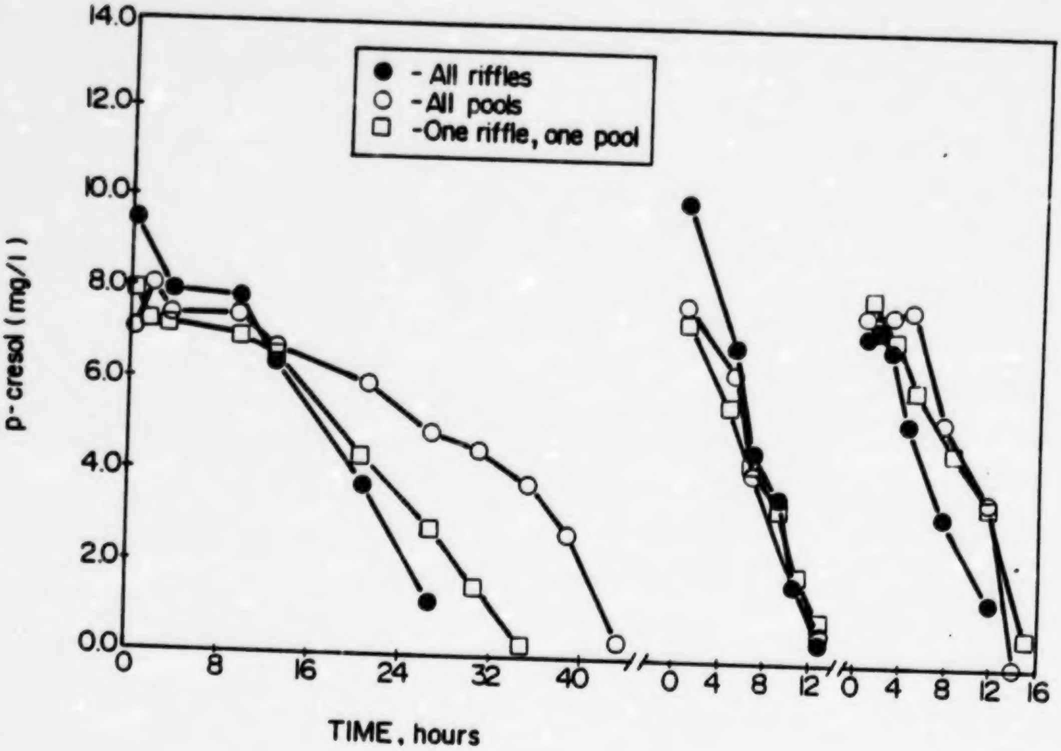

Figure 15. Rate of disappearance of p-cresol (initial concentration, 8 mg/ℓ) from three types of microcosm sets; two riffle tanks (●) two pool tanks (○) and one riffle tank/one pool tank (□). At hour 45 and 60, all three microcosms were respiked with 8 mg/ℓ p-cresol. (After Pritchard et al., 1982).

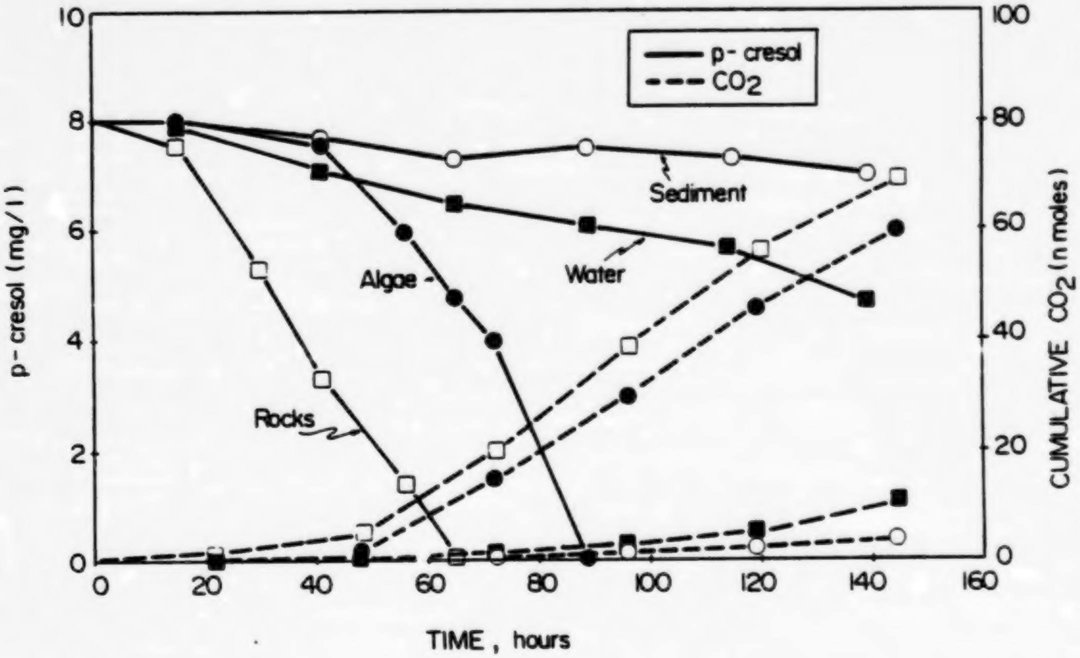

Figure 16. Disappearance (solid lines) and mineralization (dotted lines) rates of p-cresol in shake flask systems containing different components from the stream channels. For algae (Spirogyra; 162 mg wet wt. per liter), sediment (340 mg wet wt. per liter) and rocks (seven, 1.4 mg dry wt. of periphyton per rock), components were washed 3 times with sterile stream water and then incubated in sterile stream water supplemented with p-cresol. (After Pritchard and Van Veld, 1982).

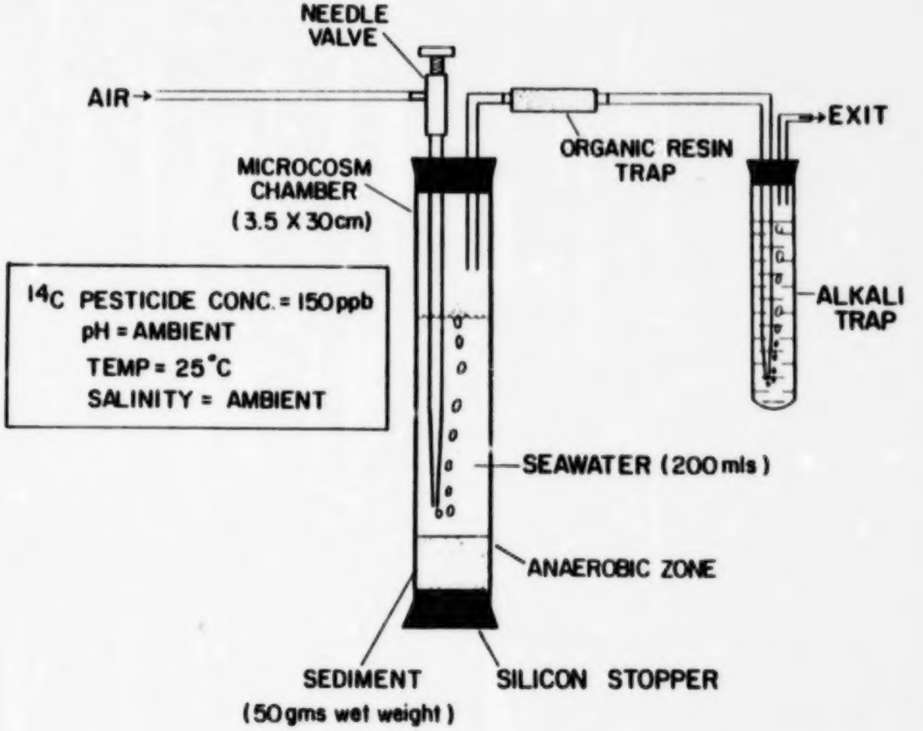
NEEDLE VALVE
AIR→
→EXIT
ORGANIC RESIN TRAP
MICROCOSM CHAMBER (3.5 X 30cm)
^{14}C PESTICIDE CONC. = 150ppb
pH = AMBIENT
TEMP = 25°C
SALINITY = AMBIENT
ALKALI TRAP
SEAWATER (200mls)
ANAEROBIC ZONE
SEDIMENT (50gms wet weight)
SILICON STOPPER

Figure 17. Schematic diagram of eco-core biodegradation test system used for biodegradation studies in estuarine salt marsh environments. (After Pritchard et al., 1979).

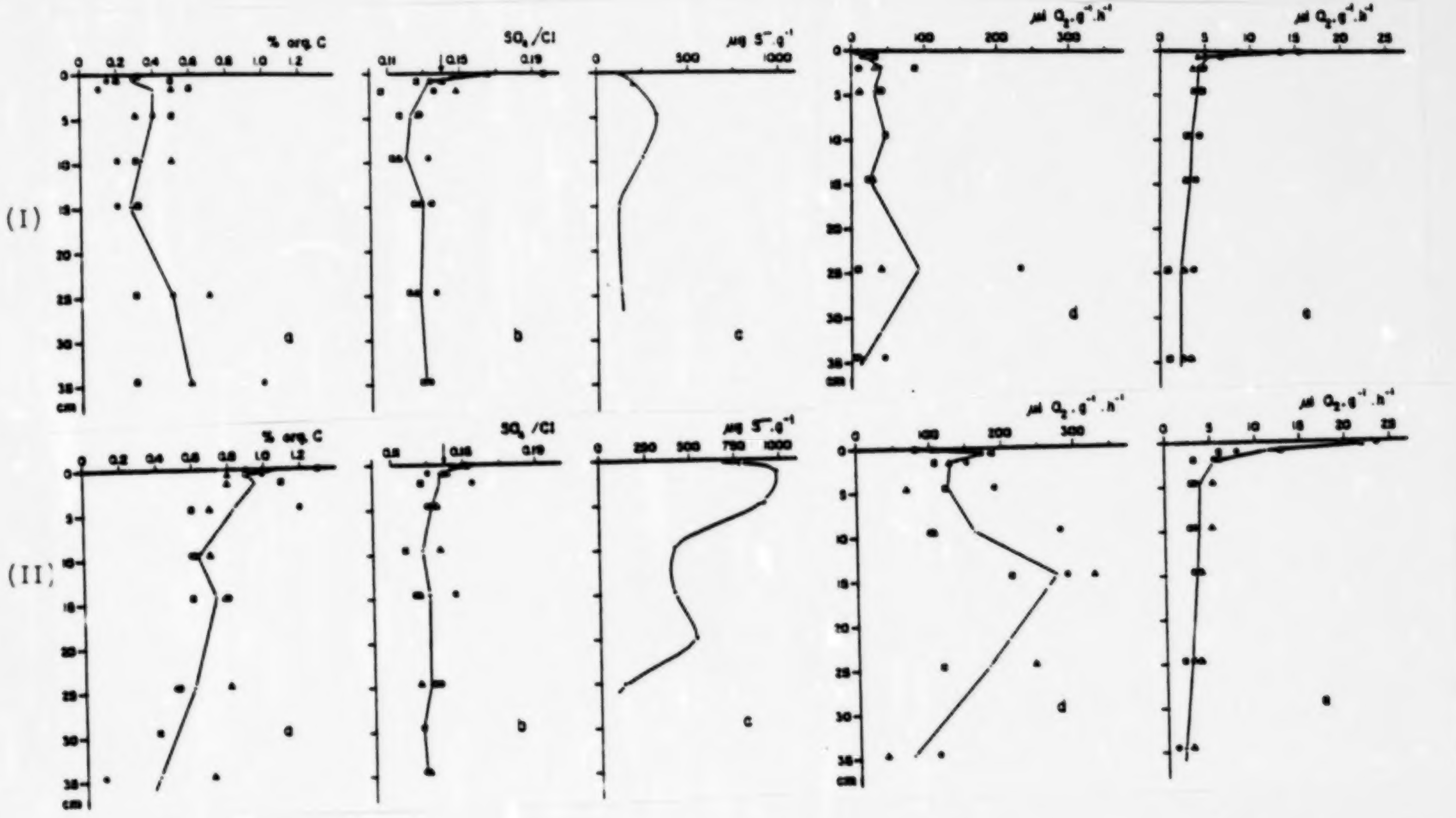

Figure 18. Vertical distributions in a natural tidal flat sediment from the Dutch Wadden Sea (I) and an artificial tidal flat system (II); a. organic carbon content (% of dry sediment); b. sulphate-chlorinity ratio in the interstitial water (arrow indicates sea water ratio); c. sulphide content (µg $S^{--}.g^{-1}$); d. oxygen utilization µl$O_2 \cdot g^{-1}.h^{-1}$). Determinations in 3 separate cores; lines connect mean values (After Vosjan and Olan Czuk-Xleyman, 1977).

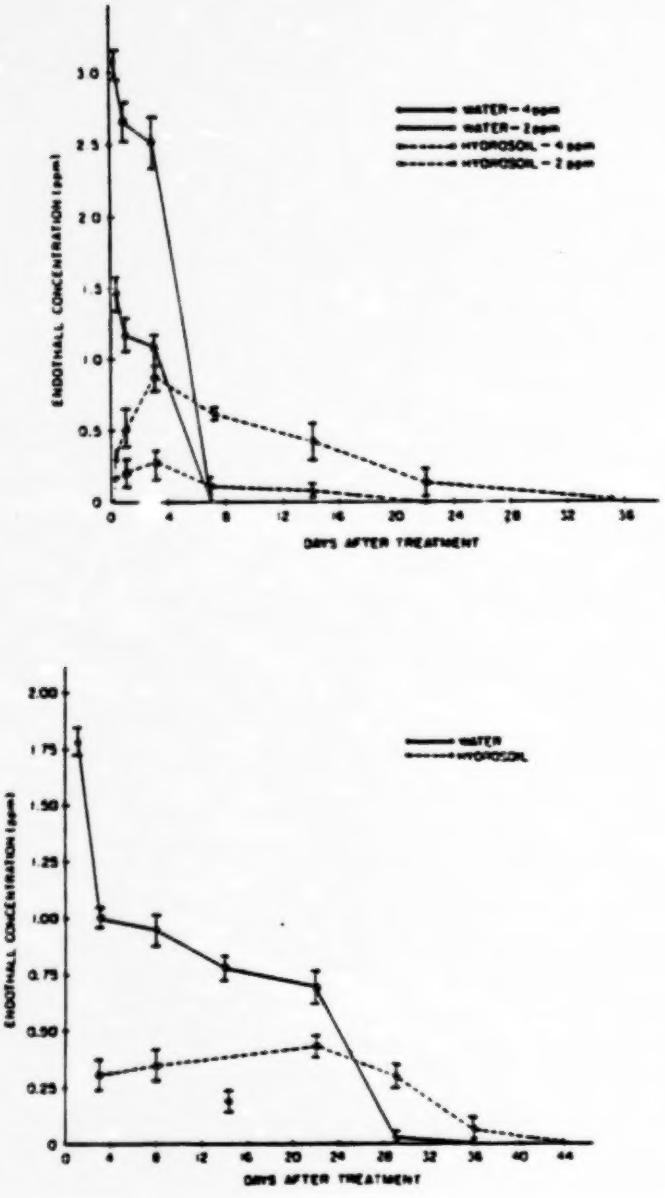

Figure 19. Endothall residues in water and hydrosoil taken from laboratory aquaria and from a farm pond. Aquaria were treated with 2 and 4 ppm of the herbicide and farm pond was treated with 2 ppm. The bars represent the range of duplicate values. (After Sikka and Rice, 1973).

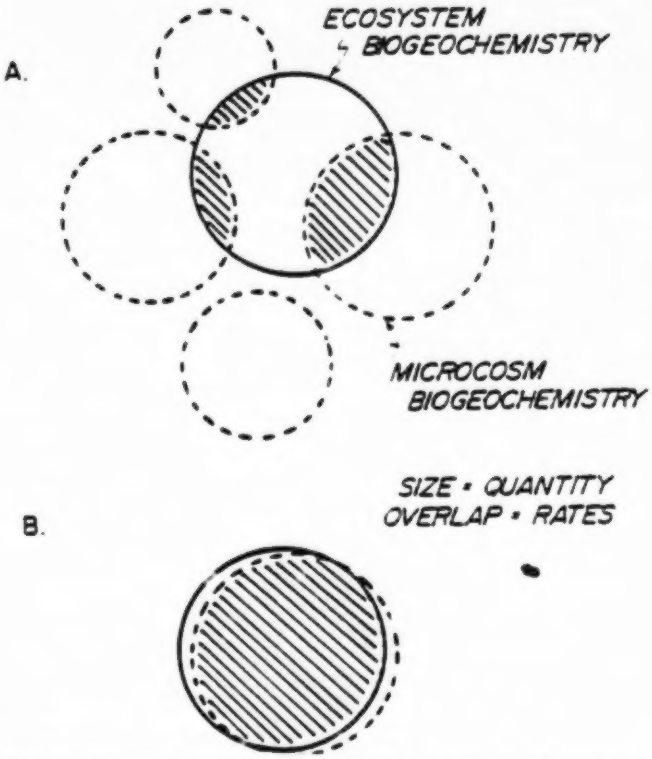
A.
ECOSYSTEM BIOGEOCHEMISTRY
MICROCOSM BIOGEOCHEMISTRY
SIZE • QUANTITY
OVERLAP • RATES
B.

Figure 20. Schematic diagram portraying possible interrelationship between the biogeochemistry of an ecosystem and various microcosms. Size of the microcosm circles indicates extent of the ecosystem's biogeochemistry housed therein. Overlap of the circles indicates similarities in rates of biogeochemical processes in microcosms and ecosystems. A and B represent possible examples of these interrelationships.

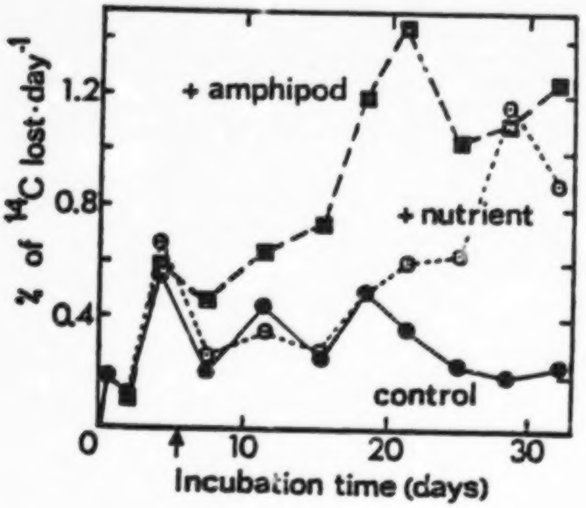

Figure 21. Daily rate of loss of ^{14}C(as $^{14}CO_2$) from labelled hay incubated at 5°C in seawater, showing the effects of addition of inorganic nutrients or of an amphipod at the time shown by the arrow. Points are means of 2 replicates. (After Harrison, 1977).

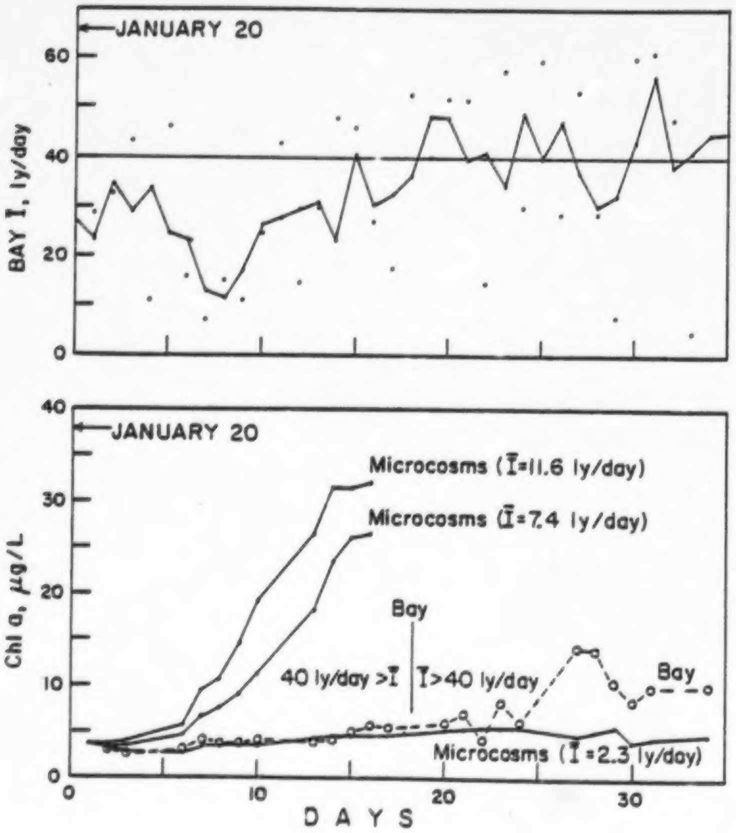

Figure 22. Chlorophyll a concentrations over time in the lower west passage of Narragansett Bay and in laboratory microcosms maintained at different light levels but with the same photoperiod as the bay. The microcosms were also kept within 1-2°C of the bay temperature. Results for the microcosms at light intensities of 25, 16, and 5 ly/day are shown. Values plotted are the means for duplicate tanks. Daily values (·) for the approximate mean light in the water column in the bay are shown, along with the 3 day moving average (--) for the period preceeding and during the bay bloom in February 1975. (From Nixon et al., 1979).

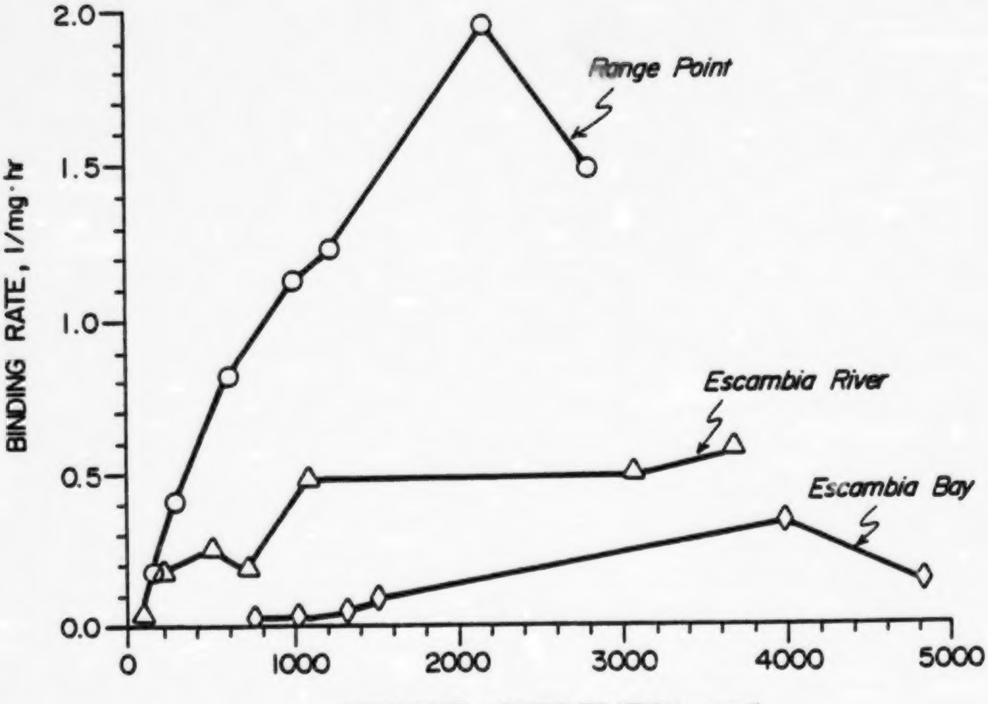

Figure 23. Effect of sediment concentration on binding of ^{14}C (ring-labeled) methyl parathion to three different sediment types taken from the Florida Gulf Coast. Binding is the amount of radioactivity which becomes associated with sediment and cannot be removed by solvent extraction. The bound radioactivity is measured by combustion and traping of $^{14}CO_2$. (After Cleveland, 1982).

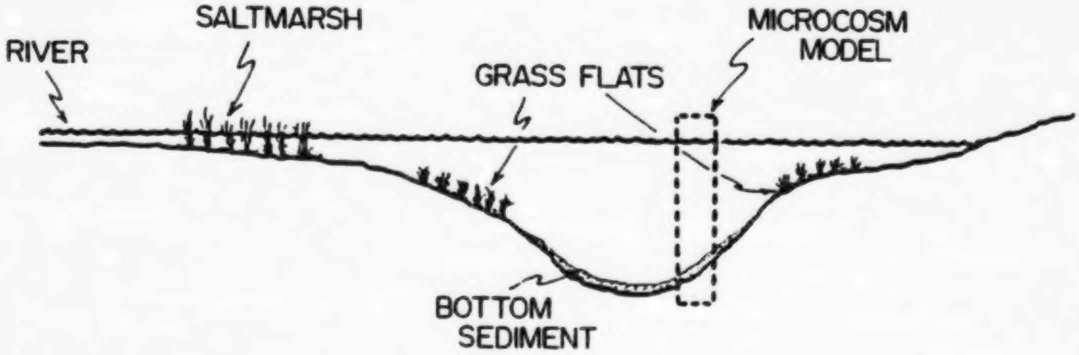
SALTMARSH
RIVER
MICROCOSM MODEL
GRASS FLATS
BOTTOM SEDIMENT

Figure 24. Diagrammatic cross section of a typical estuarine ecosystem. Dotted lined box represents portion of the ecosystem that could be modeled in a microcosm.